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A North American Strategic Plan to Prevent and Control  
Invasions of the Lethal Salamander Pathogen  
*Batrachochytrium salamandrivorans*



North American  
*Bsal* Task Force

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**North American *Bsal* Task Force**

**2022**

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Cover photo: Color-inverted photo of a Green Salamander (*Aneides aeneus*), illustrating skin lesions (white markings) that developed following experimental exposure to *Bsal*. Green Salamanders are a species of greatest conservation need in several US states and are highly susceptible to *Bsal* chytridiomycosis. Photo credit: Todd Amacker.

## Foreword

North America has a rich diversity of salamander species, with the southeastern United States being especially diverse. Salamanders are largely secretive and nocturnal and therefore are not a familiar group of vertebrates to many. However, their abundance and biomass can be very large in forest and aquatic habitats, and indeed, they perform critical roles in these ecosystems. Unfortunately, salamander species will be threatened if the chytrid fungus *Batrachochytrium salamandrivorans* arrives in North America. Evidence from Europe, where *Bsal* arrived from Asia as a naïve and deadly pathogen, shows that populations of salamanders can be extirpated when the pathogen arrives.

The North American *Bsal* Task Force was formed to confront the threat and to proactively devise a strategic plan. The Technical Advisory Committee (TAC) is the leadership group of the Task Force, and it includes members from the three countries in North America. Members of the Task Force are experts and also volunteers united in their concern for and love of amphibians. The strategic plan is a roadmap for the conservation of salamanders, which, if successful, will maintain the abundance and diversity of salamanders and the vital ecosystem functions that they provide. Of the many important elements of the strategic plan, proposed ways to keep *Bsal* out of North America are critical since mitigation methods are still being researched. There is evidence from Europe that *Bsal* was introduced into a region from salamanders acquired in the pet trade. Importantly, the TAC includes representatives from the pet trade who are willing to help devise strategies to keep the pathogen out of North America. Another aspect of the strategic plan is a rapid response template that can be customized and enacted by local officials, should *Bsal* be detected in North America, to limit or eliminate the spread of the pathogen.

We call on scientists and the public alike to recognize the threat posed by *Bsal* and to do everything possible to prevent its introduction. Salamanders, like all species, are under threat from other factors, such as habitat loss and degradation. An understanding of the abundance, diversity, and ecological value of salamanders can motivate all of us to protect them from all threats and to ensure their long-term survival.

—Reid Harris

James Madison University & Amphibian Survival Alliance  
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Marbled Newt (*Triturus marmoratus*). © Eric Isselée.

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# A North American Strategic Plan to Prevent and Control Invasions of the Lethal Salamander Pathogen *Batrachochytrium salamandrivorans*

North American *Bsal* Task Force

## Summary

Chytridiomycosis is a fungal disease of amphibians that has led to widespread mortality and extinctions. It has been considered “the greatest threat to biodiversity of any known disease” (Wake and Vredenburg 2008). In 1999, the pathogen causing this disease was described: *Batrachochytrium dendrobatidis*, or *Bd* (Longcore et al. 1999). A second *Batrachochytrium* species that causes chytridiomycosis has been discovered more recently: *B. salamandrivorans*, or *Bsal* (Martel et al. 2013). *Bsal* has caused population extirpations of the Fire Salamander (*Salamandra salamandra*) in Europe, where it appears to have been recently introduced and its distribution is broadening (Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Wagner et al. 2019, Lötters et al. 2020). Several lines of evidence support an Asian origin of *Bsal* (Martel et al. 2013, 2020; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). Susceptibility trials of salamander species native to North America revealed that some species are lethally affected, including all species evaluated in the newt family Salamandridae (Martel et al. 2014, Carter et al. 2020). Introduction of *Bsal* to North America, a hotspot of salamander diversity, could drastically reduce amphibian biodiversity and result in devastating ecosystem effects (Gray et al. 2015, Yap et al. 2015, Richgels et al. 2016, Basanta et al. 2019). To date, *Bsal* has not been detected in North America (defined as Canada, Mexico, and the United States; e.g., Govindarajulu et al. 2017; Klocke et al. 2017; Waddle et al. 2019, 2020). Since effective mitigation strategies have not been developed to combat *Bsal* in the field (but see Thomas et al. 2019 for review; Martel et al. 2020), the best threat-abatement strategy currently available is to prevent invasion and keep the pathogen from being introduced and becoming established in North America (Grant et al. 2017, Stegen et al. 2017). If *Bsal* is detected, a rapid response plan will be essential for managing and controlling its spread (Appendix 4).

In addition to these two main goals of **preventing invasion** and being able to **respond quickly** if *Bsal* were detected in North America, it is vital to develop a more comprehensive network of strategic actions. This “North American Strategic Plan to Control Invasions of *Batrachochytrium salamandrivorans*” (hereafter the “North American *Bsal* Strategic Plan”) outlines integrated, multi-pronged actions developed by the North American *Bsal* Task Force (or *Bsal* Task Force) for detecting and preventing the establishment of *Bsal* in North America. The North American *Bsal* Strategic Plan includes the following components: development of **response and control measures** to facilitate response and rapid mitigation actions; improved **diagnostic tools** to identify the pathogen with accuracy and efficiency, including a network of diagnostic laboratories able to analyze samples for *Bsal*; **research** to better understand *Bsal* effects on North American species and how to potentially safeguard susceptible species; application of **decision science** to frame and solve management problems, identify research needed to improve management, and guide data collection and use; a **surveillance and monitoring** program to maximize the potential for early detection of the fungus and documentation of its distribution and spread if found; a **data management** system to track *Bsal* surveillance in the field and captivity and to aid in planning future studies; **outreach and**

**communication** pathways for rapid dissemination of new developments; and a **clean trade program** to promote actions that reduce *Bsal* translocation in trade markets, such as the pet industry.

The *Bsal* Task Force is unique in that it is proactively designed, it is interactive among all components described above, and it includes decision science as a critical element in linking other components, especially surveillance, research, and management. The transdisciplinary approach to the North American *Bsal* Strategic Plan relies on the integration of the strategic planning of objectives and goals of the *Bsal* Task Force Technical Advisory Committee (TAC) and working groups that represent the key components outlined above. This approach has resulted in the identification of goals and actions for each component as well as an overarching Framework of Actions (below). Elements of the North American *Bsal* Strategic Plan, including the Rapid Response Plan (Appendix 4) and the Implementation Plan (Appendix 5), are intended to be updated as initial actions are implemented and new information, techniques, or priorities are identified.

### Framework of Actions

1. Prevent invasion of *Bsal* into North America by encouraging stakeholders to work toward a clean trade program for amphibians that certifies individuals in trade are free of *Bsal* infection.
2. Develop and encourage use of the Rapid Response Plan (Appendix 4 in this Strategic Plan), which can be customized to meet local needs, to contain a *Bsal* outbreak.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of *Bsal* in animal or environmental samples in a timely manner.
4. Test for the occurrence of *Bsal* in Canada, Mexico, and the United States in the field and in captivity, reduce the risk of spillover from captive to wild amphibians, and reduce the likelihood of humans playing a role in the inadvertent translocation of *Bsal* within North America.
5. Advance the understanding of the risk of *Bsal* introduction to North America and assess the invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing *Bsal* surveillance data.
6. Develop effective, scientifically justified prevention and mitigation strategies that prevent *Bsal*-associated infections and mortality.
7. As evidence-based *Bsal* response and management actions are developed, identify expedited pathways for permitting actions and facilitate regulatory processes to implement mitigation measures.
8. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners that can communicate updates on *Bsal* developments and alert the public and scientific community if *Bsal* is detected in Canada, Mexico, or the United States.

# Plan estratégico norteamericano para prevenir y controlar invasiones de *Batrachochytrium salamandrivorans*, un patógeno letal para salamandras

Grupo de trabajo *Bsal* en Norte América

## Resumen

La quitridiomycosis es una enfermedad fúngica que afecta a los anfibios y es responsable de muertes masivas y extinciones de especies en todo el mundo. Por ende, esta enfermedad es considerada la mayor amenaza a la biodiversidad (Wake and Vredenburg 2008). En 1999, se describió por primera vez al hongo *Batrachochytrium dendrobatidis*, o *Bd*, como la especie responsable de colapsos poblacionales y extinciones en anfibios (Longcore et al. 1999). Más recientemente, se ha identificado una segunda especie de hongo patógeno que también causa quitridiomycosis: *B. salamandrivorans*, o *Bsal* (Martel et al. 2013). Varias evidencias apoyan la hipótesis de que el *Bsal* tiene un origen asiático (Martel et al. 2013, 2020; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). *Bsal* ha causado extinciones locales de la salamandra de fuego *Salamandra salamandra* en Europa, en donde aparentemente fue introducido de manera reciente y en donde su rango de distribución se está expandiendo (Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Wagner et al. 2019, Lötters et al. 2020). Experimentos de susceptibilidad a *Bsal* realizados en especies de salamandras nativas de Norte América, han demostrado que *Bsal* es letal en algunas especies, incluyendo todas las especies evaluadas de tritones (familia: Salamandridae) (Martel et al. 2014, Carter et al. 2020). La introducción de *Bsal* a Norte América podría reducir de manera dramática la biodiversidad de salamandras, así como causar efectos graves en el ecosistema, ya que esta región del planeta constituye el sitio de mayor diversidad de salamandras en el mundo (Gray et al. 2015, Yap et al. 2015, Richgels et al. 2016, Basanta et al. 2019). A la fecha, *Bsal* no ha sido detectado en el continente americano. Debido a que no se han desarrollado medidas efectivas para mitigar la infección por *Bsal* en poblaciones naturales (pero ver revisión de Thomas et al. 2019, Martel et al. 2020), la mejor estrategia para disminuir los riesgos de esta enfermedad, es evitar que el patógeno se introduzca y se establezca en Norte América (Grant et al. 2017, Stegen et al. 2017). Si *Bsal* es detectado, es necesario implementar un plan de respuesta inmediato para controlar su propagación (Apéndice 4).

Si *Bsal* es detectado en Norte América es fundamental, además de **prevenir la invasión y responder de manera inmediata**, el desarrollo de una red de acciones estratégicas. Este “Plan Estratégico de Norte América para Controlar la Invasión de *Batrachochytrium salamandrivorans*” (Plan Estratégico de *Bsal* en Norte América) propone acciones integradas y multifacéticas desarrolladas por el Grupo de Trabajo *Bsal* en Norte América para detectar y prevenir el establecimiento de *Bsal* en esta región del continente americano. El Plan Estratégico de *Bsal* en Norte América se compone de los siguientes elementos: desarrollar **medidas de manejo y respuesta** que faciliten una respuesta proactiva y el desarrollo de estrategias de mitigación rápida; **herramientas mejoradas de diagnóstico** para detectar a *Bsal* con precisión y eficiencia, incluyendo la existencia de una red de laboratorios de diagnóstico capacitados para detectar al patógeno; **investigación** para entender a profundidad los efectos de *Bsal* sobre las especies de anfibios de Norte América y cómo salvaguardar a las especies susceptibles; **un marco de apoyo científico** para dar contexto y resolver problemas de manejo, identificar la investigación requerida para mejorar el manejo, así como guiar la colecta y uso de datos; un



**programa de vigilancia y monitoreo** para maximizar las probabilidades de una detección temprana del hongo y documentar su distribución y propagación en caso de ser detectado; un **sistema de manejo de datos** para rastrear el monitoreo de *Bsal* en el campo y en cautiverio, y ayudar en la planeación de estudios futuros; **vías de comunicación y divulgación** para la diseminación rápida de nuevos avances o descubrimientos; y un **programa de comercio limpio** para promover acciones que reduzcan la translocación de *Bsal* en el comercio de especies, como es el caso de la industria de mascotas.

El Grupo de Trabajo *Bsal* se distingue por su naturaleza proactiva, y por un diseño interactivo de los elementos que lo componen. El Grupo se apoya en decisiones basadas en la ciencia como un elemento crítico que enlaza a todas sus actividades, en particular en lo que se refiere a vigilancia, investigación y manejo. El enfoque transdisciplinario del Plan Estratégico de *Bsal* en Norte América depende de la integración del Comité de Asesoría Técnica (TAC, por sus siglas en inglés), que facilita la comunicación entre los diferentes sub-grupos de trabajo que integran el Grupo de Trabajo *Bsal*. Esta integración se refleja en los elementos clave incluidos en este documento, incluyendo los objetivos y metas del TAC, los cuales pueden resumirse en el Plan de Acción (abajo). Algunos elementos del Plan Estratégico de *Bsal* en Norte América, incluyendo el Plan de Respuesta Rápida (Apéndice 4) y el Plan de Implementación (Apéndice 5), serán actualizados conforme se implementen sus recomendaciones y conforme surjan nuevas fuentes de información, técnicas o prioridades.

## Plan de acción

1. Prevenir la invasión de *Bsal* a Norte América incentivando a los tomadores de decisiones para desarrollar un programa de comercio limpio para anfibios, el cual certifique que los organismos estén libres de la infección por *Bsal*.
2. Desarrollar e incentivar el uso de un plan de respuesta para contener un brote de *Bsal*, el cual está contenido en el Plan Estratégico y que puede adaptarse a las necesidades locales.
3. Desarrollar una red de laboratorios para diagnóstico que pueda realizar de manera eficiente pruebas estandarizadas para detectar la presencia de *Bsal* en muestras de anfibios o muestras ambientales.
4. Evaluar la ocurrencia de *Bsal* en Estados Unidos, Canadá y México en el campo y en cautiverio, reducir el riesgo de propagación de *Bsal* de organismos en cautiverio hacia organismos silvestres, y reducir la probabilidad de que los humanos acarreen *Bsal* de manera inadvertida a través de Norte América.
5. Avanzar en el entendimiento sobre los riesgos de la introducción de *Bsal* a Norte América y evaluar este riesgo mediante análisis de ciencia aplicada, investigación, y generación de un repositorio para incorporar y manejar datos de monitoreo de *Bsal*.
6. Desarrollar estrategias de mitigación y prevención que sean efectivas y sustentadas científicamente para prevenir la mortalidad y las infecciones asociadas a *Bsal* en anfibios.
7. Identificar vías expeditas para permitir acciones, facilitar procesos regulatorios e implementar estrategias de mitigación, mientras se desarrollan acciones de manejo y respuesta con base en evidencias científicas.

8. Trabajar con colaboradores y colegas para compilar y diseminar resultados de monitoreos e investigación sobre *Bsal* por medio de redes sociales, portales de bases de datos disponibles, y artículos o notas periodísticas.
9. Crear una red de colegas que puedan comunicar las actualizaciones en el tema de *Bsal* y alertar al público y a la comunidad científica en caso de que *Bsal* sea detectado en Estados Unidos, Canadá o México.

# Plan stratégique nord-américain pour la prévention et le contrôle des invasions de *Batrachochytrium salamandrivorans*, un pathogène mortel pour les salamandres

Le groupe de travail North American *Bsal* Task Force

## Résumé

La chytridiomycose est une maladie fongique des amphibiens qui cause dans une large mesure la mortalité et l'extinction. Elle est considérée comme « la plus grande menace à la biodiversité de toutes les maladies connues » (Wake et Vredenburg 2008). Le pathogène qui cause cette maladie a été identifié en 1999 : *Batrachochytrium dendrobatidis*, ou *Bd* (Longcore et coll. 1999). Une seconde espèce de *Batrachochytrium* causant la chytridiomycose a été découverte plus récemment : le *B. salamandrivorans*, ou *Bsal* (Martel et coll. 2013). *Bsal* cause la disparition de populations de salamandres tachetées *Salamandra salamandra* en Europe, où il semble avoir été récemment introduit et dont la distribution semble s'étendre (Spitzen-van der Sluijs et coll. 2016, Stegen et coll. 2017, Wagner et coll. 2019, Lötters et coll. 2020). Plusieurs éléments de preuve indiquent que *Bsal* serait d'origine asiatique (Martel et coll. 2013, 2020; Laking et coll. 2017; Nguyen et coll. 2017; Yuan et coll. 2018). Des essais de sensibilité des espèces de salamandres originaires d'Amérique du Nord ont révélé que certaines espèces sont mortellement affectées, et parmi elles toutes les espèces évaluées dans la famille de tritons salamandridés (Martel et coll. 2014, Carter et coll. 2020). Si *Bsal* était introduit en Amérique du Nord, haut lieu de la diversité des salamandres, cela pourrait réduire considérablement la biodiversité des amphibiens et avoir un effet dévastateur sur l'écosystème (Gray et coll. 2015, Yap et coll. 2015, Richgels et coll. 2016, Basanta et coll. 2019). À ce jour, *Bsal* n'a pas été détecté en Amérique du Nord (terme désignant le Canada, le Mexique et les États-Unis; Govindarajulu et coll. 2017; Klocke et coll. 2017; Waddle et coll. 2019, 2020). Puisque des stratégies d'atténuation efficaces n'ont pas été mises en place pour combattre *Bsal* sur le terrain (mais voir Thomas et coll. 2019 pour une revue de la littérature; Martel et coll. 2020), la meilleure stratégie de réduction de la menace présentement disponible serait de prévenir l'invasion et d'empêcher que ce pathogène ne soit introduit et ne s'établisse en Amérique du Nord (Grant et coll. 2017, Stegen et coll. 2017). S'il est détecté, un plan de réponse rapide sera essentiel pour la gestion et le contrôle de sa propagation (Annexe 4).

En plus de ces deux objectifs principaux de **prévention de l'invasion** et de capacité de **réponse rapide** si *Bsal* est détecté en Amérique du Nord, il est essentiel de mettre sur pied un réseau plus complet d'actions stratégiques. Le "North American Strategic Plan To Control Invasions of *Batrachochytrium salamandrivorans*" (North American *Bsal* Strategic Plan) détaille des actions intégrées sur plusieurs fronts et mises en marche par la North American *Bsal* Task Force pour détecter et prévenir l'établissement de *Bsal* en Amérique du Nord. Le North American *Bsal* Strategic Plan comprend les éléments suivants : la mise en œuvre de mesures de **réponse et contrôle** dynamiques déclenchant des actions d'atténuation rapides; de meilleurs **outils diagnostiques** permettant d'identifier le pathogène avec précision et efficacité, dont un réseau de laboratoires diagnostiques capables d'analyser des échantillons de *Bsal*; de la **recherche** pour mieux comprendre les effets de *Bsal* sur les espèces nord-américaines et peut-être même protéger les espèces sensibles; l'application de la **science décisionnelle** pour définir et régler les problèmes de gestion, pour déterminer les besoins en recherche visant à améliorer la

gestion et pour diriger la collecte et l'utilisation des données; un programme de **surveillance et de contrôle** pour maximiser le potentiel de détection précoce du champignon et pour documenter son éventuelle distribution et étendue; un système de **gestion des données** pour retracer les activités de surveillance de *Bsal* sur le terrain et en captivité, et aider à planifier les prochaines études; des voies de **communication et de sensibilisation** pour permettre de faire circuler les nouveaux éléments d'information; et un **programme de « commerce propre »** pour faire la promotion d'actions diminuant la translocation de pathogènes sur les marchés, comme le marché des animaux de compagnie.

Le groupe *Bsal* Task Force est une initiative unique, car elle est dynamique, et tous les éléments décrits ci-dessus sont interactifs, avec la science décisionnelle comme point critique pour lier les autres éléments, dont la surveillance, la détection et la gestion. L'approche interdisciplinaire du North American *Bsal* Strategic Plan dépend de l'intégration de la planification stratégique des buts et objectifs du *Bsal* Task Force Technical Advisory Committee (TAC) and working groups, c.-à-d. les éléments clés décrits ci-dessus. Cette approche a mené à la détermination des buts et des actions pour chaque élément ainsi que pour le Cadre d'actions (voir ci-dessous). Les éléments du North American *Bsal* Strategic Plan, dont un plan de réponse rapide (Annexe 4) et un plan d'implantation (Annexe 5), devront être mis à jour quand les premières actions seront entreprises et que de nouvelles données, techniques ou priorités seront déterminées.

## Cadre d'actions

1. Prévenir l'invasion de *Bsal* en Amérique du Nord en encourageant les parties prenantes à établir un programme de « commerce propre » pour les amphibiens qui certifie que les individus vendus n'ont pas d'infection au *Bsal*.
2. Mettre sur pied et promouvoir l'utilisation du plan de réponse décrit dans le plan stratégique, qui peut être adapté aux besoins locaux pour contenir une éclosion de *Bsal*.
3. Mettre sur pied un réseau de laboratoires diagnostiques capables d'exécuter des tests validés permettant de détecter à temps la présence de *Bsal* chez un animal ou dans un échantillon environnemental.
4. Dépister la survenue éventuelle de *Bsal* aux États-Unis, au Canada et au Mexique sur le terrain et en captivité, réduire le risque de débordement des amphibiens captifs vers les sauvages et réduire la possibilité que les humains jouent un rôle dans le transfert par inadvertance de *Bsal* en Amérique du Nord.
5. Améliorer la compréhension du risque lié à l'introduction de *Bsal* en Amérique du Nord et évaluer le risque d'invasion des amphibiens indigènes par ce pathogène mortel grâce à des analyses de science décisionnelle, à de la recherche et à la mise sur pied d'un référentiel commun pour colliger et gérer les données de surveillance de *Bsal*.
6. Grâce au soutien de la science, concevoir des stratégies de prévention et d'atténuation efficaces empêchant les infections et la mortalité liées au *Bsal*.
7. Pendant que des interventions et des cadres de gestion factuels de *Bsal* sont mis sur pied, en déterminer les voies d'accès privilégiées et aider au processus réglementaire menant à l'implémentation de mesures d'atténuation.

8. Travailler avec les partenaires pour colliger et distribuer les résultats de la recherche et de la surveillance à travers les réseaux sociaux, les bases de données accessibles en ligne et les articles des bulletins d'information.
9. Établir un réseau de partenaires capables de fournir des mises à jour de l'information concernant *Bsal* et d'alerter le public et la communauté scientifique si *Bsal* est détecté aux États-Unis, au Canada ou au Mexique.

## Strategic Plan Overview: Vision, Purpose, and Components

The vision of the North American *Bsal* Strategic Plan is to help safeguard North American amphibians from a novel and emerging pathogen. Specifically, the risk is that the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) will spread to North America, where it is not currently known to occur, and will cause population losses of amphibians from the skin disease chytridiomycosis. The plan's purpose is to outline a comprehensive approach to reduce the likelihood of *Bsal* establishment in North America, develop and evaluate proactive management actions to reduce the risk of *Bsal* introduction and establishment, and develop actions to combat the disease if it were to be found. It is a multipronged strategy that engages a community of researchers, natural resource managers, and partners from the public sector in Canada, Mexico, and the United States.

The North American *Bsal* Strategic Plan includes background information on *Bsal* and the ecological significance of salamanders in ecosystems and a brief review of policy options aimed at preventing *Bsal* introduction to North America. The North American *Bsal* Task Force (or *Bsal* Task Force) is described, including the strategic goals of the oversight group (the Technical Advisory Committee [TAC]) and the following working groups: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade. The Rapid Response Plan Template, which provides guidance for field or captive situations if *Bsal* were to be suspected in North America, is included as Appendix 4. The *Bsal* Implementation Plan, which outlines more specific goals and priorities of the TAC and working groups, is included as Appendix 5. Both Appendices 4 and 5 are expected to adapt over time to new information and updates will be posted on relevant websites (e.g., [salamanderfungus.org](http://salamanderfungus.org)).

At the time of this writing, *Bsal* is not known to occur in North America and strategies included herein are consistent with the high-alert condition of *Bsal* being yet undetected or rare in North America.



White-spotted Slimy Salamander (*Plethodon cylindraceus*). © Alberto López.

## I. Background

### *Batrachochytrium salamandrivorans*

*Bsal* is a member of the Chytridiomycota, which is an early evolved group of fungi characterized by production of zoospores with a single posteriorly directed flagellum (Powell 2016). Members of this group are largely decomposers; however, the two species of the genus *Batrachochytrium* have adaptations to infect amphibians, growing and reproducing in their skin. *Batrachochytrium dendrobatidis* (*Bd*) is known to infect frogs, salamanders, and caecilians, and has caused widespread population declines and extinctions, especially of frog species (Lips et al. 2006, Skerratt et al. 2007, Scheele et al. 2019). *Bd* and/or its metabolites are suspected of causing deleterious effects on crayfish, but definitive evidence of infection in invertebrates is lacking (McMahon et al. 2013). Among amphibians, some species can carry *Bd* infections without showing signs of the disease chytridiomycosis (e.g., Bullfrogs [*Rana catesbeiana*]: Daszak et al. 2004), and some species are resistant to infection (Appendix 1). *Bsal* is a recently described, closely related congener of *Bd* that has caused salamander population declines and extirpations in Europe (Stegen et al. 2017, Lötters et al. 2020, Martel et al. 2020).

Understanding the life history of the *Bsal* pathogen is important, as it relates to its modes of transmission and differs somewhat from that of *Bd*. In both *Bd* and *Bsal*, reproduction consists of the asexual production of spores (Robinson et al. 2020). A sexual stage has not been observed in *Bsal* or *Bd* but is common in other chytrids (Powell 2016). Evidence of past sexual reproduction (hybridization) has been detected in *Bd* (Schloegel et al. 2012, Jenkinson et al. 2016). *Bsal* has two types of spores, whereas *Bd* has one. Like all other members of the Chytridiomycota, *Bsal* produces flagellated zoospores, but it also produces unflagellated encysted spores (Stegen et al. 2017). Stegen et al. (2017) reported that the flagellated zoospores swim toward their potential host and can be consumed by micropredators, which are predominantly types of zooplankton. That study also found that encysted *Bsal* spores float on the water's surface, can persist in forest soils for a time, and adhere to amphibian hosts as well as to the feet of waterfowl, which could lead to widespread dispersal. When in water, the encysted spores were viable and infective for Fire Salamanders (*Salamandra salamandra*) for at least 31 days and were more resistant to predation by zooplankton. Transmission of *Bsal* through contaminated forest soils occurred for up to 48 hours after the soil had been in contact with an infected salamander. Stegen et al. (2017) also reported that *Bsal* DNA could be detected from contaminated forest soils even after 200

days. The existence of both flagellated and encysted spores is likely to increase within-population transmission rates above what would occur with flagellated zoospores alone (Stegen et al. 2017).

Amphibian host species that are lethally infected and species that are tolerant of infection (carriers) can both contribute to transmission of the pathogen. Species that carry the infection but do not succumb constitute a reservoir for the pathogen. So far, only salamander species have been found to be lethally infected by *Bsal*, whereas both salamander and frog species can be carriers of *Bsal* (Martel et al. 2014, Nguyen et al. 2017, Stegen et al. 2017). A recent study found a surprisingly limited rate of dispersal of *Bsal* among populations, perhaps due to a fragmented landscape of suitable habitat types for salamanders (Spitzen-van der Sluijs et al. 2018). In the field, we lack a full understanding of *Bsal* transmission pathways, which are likely to have context-dependent dynamics. For example, transmission may vary with habitat heterogeneity, amphibian community composition, and *Bsal* physiological ecology.

*Bsal* evolved in Asia, where the lack of lethal infections suggests a long co-evolutionary history that has led to resistance or tolerance in native amphibian species (Laking et al. 2017). *Bsal* was recently discovered in Europe (Martel et al. 2013), and it appears to be spreading (e.g., Spitzen-van der Sluijs et al. 2016, Lötters et al. 2020, Martel et al. 2020). Infections were first observed in the Netherlands, followed by the discovery of *Bsal* in Belgium and Germany. Most recent evidence suggests that Germany may be the site of the index case for introduction of *Bsal* into Europe (Lötters et al. 2020). The lethal effect of *Bsal* on some European amphibian species suggests a recently arrived pathogen that has encountered naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations, movement of spores by other wildlife and humans, and release of infected individuals once held in captivity (Nguyen et al. 2017, Yuan et al. 2018, Martel et al. 2020). Spread of *Bsal* between continents is likely due to importation of infected species from locations where *Bsal* is endemic (Nguyen et al. 2017). Indeed, anurans from Asia infected with *Bsal* have been found in a pet store in Germany (Nguyen et al. 2017, Yuan et al. 2018). In addition, *Bsal* has been found on salamander species in China that are frequently imported. These findings suggest a role of trade markets in the between-continent spread of *Bsal*. In particular, the discovery that anurans can be infected opens up the possibility that trade in frogs for food, research, and pets can lead to between-continent dispersal.

### **Distribution of *Bsal* in the wild and in captivity**

*Bsal* has been found in the wild and in captivity in Asia and Europe (Appendix 2). *Bsal* has infected over 20 amphibian species in the wild (Martel et al. 2013, 2014; Spitzen-van der Sluijs et al. 2016; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018) and at least nine species in captivity (Martel et al. 2014, Cunningham et al. 2015, Nguyen et al. 2017, Fitzpatrick et al. 2018, Sabino-Pinto et al. 2018, Yuan et al. 2018). In the wild, most infected species have been in the family Salamandridae (17 infected species). One frog species found to be infected in the wild was the Many-webbed Fire-bellied Toad (*Bombina microdeladigitora*) (Nguyen et al. 2017). This species is closely related to the Oriental Fire-bellied Toad (*Bombina orientalis*), which is widely imported into the United States in pet trade markets. An enormous number of Oriental Fire-bellied Toads (3.5 million individuals) were imported into the United States between 2001 and 2009. Nguyen et al. (2017) concluded that *Bsal* was vectored into the wild in Europe via the pet trade.





Figure 1. Skin shedding on the foot of a *Bsal*-infected Eastern Newt (*Notophthalmus viridescens*). Small, circular lesions, a characteristic sign of *Bsal* chytridiomycosis, can be seen on the base of the foot. Photo credit: Todd Amacker.

### Susceptibility of amphibian species to *Bsal* infection

The *Bsal* case definition describes the presentation of the disease *Bsal* chytridiomycosis (White et al. 2016) resulting from *Bsal* infection (Fig. 1). However, disease signs are not apparent in all infections. Four terms have been used to classify host response to *Bsal* (Martel et al. 2014; F. Pasmans, Ghent University, Belgium, pers. comm. to D. H. Olson, 23 June 2020): 1) lethal = infection that invariably (as far as we know) results in death after a single exposure to a low dose (e.g., 1000 zoospores); 2) susceptible = infection resulting in variable infection and disease dynamics, depending on dose of infection (and probably a large number of other determinants); 3) tolerant = infection never resulting in disease or mortality, given current knowledge; and 4) resistant = no natural infections known and no infection in laboratory trials. In the absence of knowledge of within-species variation in *Bsal* response, these terms have been used to classify species-level responses (Martel et al. 2014).

The effects of *Bsal* infection vary among amphibian species. Some species, including those in the newt family Salamandridae, have been reported to be lethally infected by *Bsal* (Martel et al. 2014; Appendix 1). The Fire Salamander is highly susceptible to *Bsal*; indeed, once *Bsal* enters a population of Fire Salamanders, extirpation of the population can occur rapidly (Stegen et al. 2017). However, other salamander species, such as the Alpine Newt (*Ichthyosaura alpestris*), appear to be tolerant of infection, in the sense that they can carry an infection without experiencing morbidity. Frog species, such as the Common Midwife Toad, *Alytes obstetricans*, have also been reported to be either resistant to or tolerant of *Bsal* infections. Unpublished data on susceptibility trials in the laboratory also have indicated that some amphibian species are resistant to infection, some species carry infections but do not succumb, and some species are lethally affected (Appendix 1). So far, nine species of salamanders are known to develop

chytridiomycosis from *Bsal* and succumb, especially when infected at a high dose ( $10^6$  zoospores). The finding, both in nature and in the laboratory, that frog species can carry infections demonstrates that the host range of *Bsal* is greater than initially thought based on the work of Martel et al. (2014).

Importantly, the Siberian Salamander (*Salamandrella keyserlingii*) has been found infected with *Bsal* in the wild (Martel et al. 2014). Given that *Salamandrella* is a very early-evolved genus within the order Caudata, this finding suggests that the ability to be infected with *Bsal* is an ancestral trait and that, unless shown otherwise, it is prudent to assume that all salamander species can be infected with *Bsal*.

### **Risk models**

Three studies have explored the regions in North America that are most likely to be affected by the arrival of *Bsal* (Yap et al. 2015, Richgels et al. 2016, Basanta et al. 2019). Yap et al. (2015) used a *Bsal* habitat suitability model combined with a host-species richness map to identify four zones of high risk in North America: the southeastern United States, the western United States, the south coast of British Columbia, and the highlands of central Mexico. The model developed by Richgels et al. (2016) used habitat suitability for *Bsal* and host richness in the United States and included risk of introduction from the pet trade. Their model predicted three zones of high risk: the Pacific coast, the southern Appalachian Mountains, and the mid-Atlantic region. These models assumed equal susceptibility of host species and had broadly similar conclusions. Basanta et al. (2019) used a habitat suitability model for *Bsal* and salamander distributions in Mexico to identify high-risk zones and potential hotspot areas for surveillance. This model predicted areas of the Trans-Mexican Volcanic Belt, the Sierra Madre del Sur, the Sierra Madre Oriental, northern Oaxaca, the Mexican Gulf, and the Yucatán Peninsula as risk zones suitable for *Bsal* in Mexico, and it identified 13 hotspots with both high salamander diversity and suitability for *Bsal*. Current research into susceptibility of host species may refine these three models, which can indicate areas to focus *Bsal* surveillance efforts.

### **Ecological importance of salamanders**

Salamanders play a vital role in aquatic and terrestrial ecosystems. They can be important in energy flow through these ecosystems and in suppression of leaf litter decomposition in terrestrial ecosystems, which functions to sequester carbon from the atmosphere. They can also be important as keystone species that affect ecosystem biodiversity. Dramatic declines and extinctions of salamander species in North America could, therefore, have important negative effects on both terrestrial and aquatic ecosystems.

In forested areas of North America, the population density of terrestrial salamander species can be very high. Recent estimates that consider the detection rate of a species indicate that surface populations can be <20% of the total population (Bailey et al. 2004, Semlitsch et al. 2014). For example, if estimates are adjusted accordingly, then population densities of the Eastern Red-backed Salamander (*Plethodon cinereus*) were over  $1/\text{m}^2$  in New Hampshire's Hubbard Brook Experimental Forest (Burton and Likens 1975a) and were up to  $11/\text{m}^2$  in Virginia's Shenandoah National Park (Jaeger 1979). The population density of a related species, the Southern Red-backed Salamander (*Plethodon serratus*), was estimated to be about  $1/\text{m}^2$  in Missouri (Semlitsch et al. 2014). Even using estimates based on surface counts, the total biomass

of salamanders was estimated to be 2.5 times the biomass of all breeding birds and equal to that of small mammals in the Hubbard Brook Forest (Burton and Likens 1975a).

The large biomass of salamanders in forest ecosystems has several important implications. First, energy flow through or storage in salamanders can be large. In the Hubbard Brook Forest ecosystem in New Hampshire, it was estimated that salamanders consume 5.8 kcal/m<sup>2</sup> each year (Burton and Likens 1975b). The total amount of energy contained in soil invertebrates in a hardwood forest was estimated as 5.04 kcal/m<sup>2</sup>. Thus, salamanders' food requirements would require a complete turnover in the soil invertebrate community each year, although salamanders also can consume prey found above ground. Further, it has been suggested that predation is not a significant source of mortality for woodland salamanders in the genus *Plethodon* (Hairston 1987). If so, then a large fraction of primary production is entering the salamander community through a pathway that starts with leaf litter and proceeds to soil invertebrates and on to salamanders. Thus, salamanders can act as an important store of energy in the ecosystem and, as such, might dampen fluctuations in energy flow. Elimination of a large fraction of terrestrial salamanders could magnify stochastic fluctuations in energy flow and would release soil invertebrates from predation, which could lead to large population sizes of these invertebrates.

Increased population sizes of soil invertebrates caused by reductions in woodland salamander populations could have drastic effects on CO<sub>2</sub> release into the atmosphere. An increase in leaf-shredding soil invertebrates and the resulting increase in leaf fragments could facilitate an increase in microbial decomposers, increasing total microbial respiration as a result. Three studies have demonstrated that total leaf litter decomposition is suppressed when woodland salamanders in control plots are not experimentally removed (Wyman et al. 1998, Best and Welsh 2014, Hickerson et al. 2017). Wyman (1998) found that salamanders in New York suppressed decomposition by 11 to 17%, thereby reducing soil carbon release to the atmosphere (i.e., by their predation of soil invertebrates, these salamanders affect carbon sequestration). Another study found a similar effect and estimated that, across its range in California, the species *Ensatina eschscholtzii* could prevent 72.3 metric tons of carbon from entering the atmosphere each year (Best and Welsh 2014). Although additional replication of these studies is required, they suggest that large declines of terrestrial salamanders could release large amounts of CO<sub>2</sub> into the atmosphere that otherwise would be stored in the soil ecosystem, and this release would have concomitant effects on climate change.

Furthermore, many amphibians have a key ecological functional role in the transportation of reciprocal subsidies between aquatic and terrestrial ecosystems (Davic and Welsh 2004). As eggs are deposited in aquatic habitats and larvae develop there, they accrue aquatically derived nutrients in their body mass. These energetic subsidies are transported to terrestrial ecosystems upon metamorphosis for many species, including many salamanders. In terrestrial ecosystems, metamorphic and adult amphibians continue to grow, accruing terrestrial subsidies that are later brought back to aquatic habitats upon breeding. Such reciprocal subsidies effectively link aquatic and terrestrial ecosystem energetics.

Salamanders are keystone species in temporary pond ecosystems, meaning that their removal will have important impacts on the ecosystem. Studies from the 1980s in experimental ponds demonstrated that removal of the keystone species the Eastern Newt (*Notophthalmus viridescens*) caused changes in the anuran and zooplanktonic community structure (Morin 1983, Morin et al. 1983, Wilbur et al. 1983). Newts preyed upon the competitively dominant tadpole species, which allowed the persistence of competitively weaker species, such as the Spring Peeper (*Pseudacris crucifer*)—a dynamic that would change in the absence of newts. Based on

recent *Bsal* susceptibility trials (Longo et al. 2019), the introduction of *Bsal* to North America could decimate newt populations across their range, which could have irreversible cascading effects on biotic communities, and associated changes in energy transport among ecosystems are a likely consequence.



Eastern Newt (*Notophthalmus viridescens*). © Alberto López.

## II. Policy Review

Strategic planning to forestall *Bsal* emergence in North America intersects a variety of policy arenas. Regulatory processes may be intertwined with development of proactive measures to reduce the likelihood of *Bsal* transmission to North America (and within North America, should it be detected) and with use of reactive mitigations to control a known *Bsal* occurrence, should one be detected. Overall, a range of actions have been identified, each with policy implications: 1) restrictions on importation of *Bsal*-susceptible amphibian species; 2) protocols to ensure that any imported amphibians are free of the pathogen; 3) testing of amphibians already in captivity in North America and treatment of infected individuals and associated enclosures and fomites to remove *Bsal*; 4) a suite of mitigations for field use in response to *Bsal* detection, following lessons learned by Bosch et al. (2015) and Martel et al. (2020) as well as research findings by Bernard and Grant 2020; and 5) field and laboratory biosecurity measures to reduce inadvertent human-mediated pathogen transmission.

Relative to the first action, concerns over *Bsal* have led to the implementation of partial to complete restrictions on amphibian imports in some nations, such as Canada, Switzerland, and the United States. Canada requires a permit for the importation of all salamanders whether live or dead (CBSA 2018). The United States has enacted an interim rule of the Lacey Act that banned importation of 201 species of salamanders (USFWS 2016). So far, Mexico has not banned any amphibian imports, and the country has trade programs with Asia and the United States but not with Europe. In addition, in 2015, the Pet Industry Joint Advisory Council (PIJAC) in the United States recommended a temporary and immediate voluntary trade moratorium of imports of Asian salamander species that were known to carry *Bsal* until such time as effective testing and treatment regimens could be developed and distributed ([Pet Product News](#) 2015). PIJAC of Canada also supported a temporary moratorium on importation of Asian-native Fire Belly and Paddle-tail Newts (*Cynops* spp. and *Pachytriton labiatus*, respectively), which might transmit *Bsal* ([PIJAC Canada](#) 2016).

The second action noted above, testing and treating *Bsal*-infected animals in international trade markets prior to import, has been addressed recently in Europe: the European Union (EU) enacted legislation to create a clean trade program for salamander species (EU 2018). This EU action can serve as a model for clean trade programs in other countries and can be a model for broader legislation that could apply to pathogens of all wildlife species. Also, the World Organization for Animal Health (OIE) has listed *Bsal* as an OIE-notifiable disease (OIE and

IUCN 2014, [OIE 2020a](#)). This status requires OIE member countries to provide animal health certificates to accompany imports of domesticated animals that are potential hosts of OIE-listed pathogens, but evidence of pathogen-free shipments is not required for wildlife in many countries, including the United States. In addition, OIE member countries are supposed to report OIE-listed pathogen detections, should they occur. Further development of these proactive measures, including establishing a clean trade program and requiring pathogen-free certificates for import into North America, warrants additional consideration for broader implementation (see Clean Trade Working Group section below).

Relative to the third action, surveillance of captive animals has been conducted to a limited extent in North America, with no known *Bsal* detections to date (e.g., British Columbia, Canada: Govindarajulu et al. 2017; United States: Klocke et al. 2017). Additionally, the national Disease Task Team of Partners in Amphibian and Reptile Conservation (PARC) has implemented a “Herpetofaunal Disease Alert System” (HDAS) where anyone can send an alert email ([herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org); Gray et al. 2018) if they observe an amphibian or reptile die-off related to apparent disease causes. This alert applies to both captive and field situations, and alerts have been received for both. For example, captive alerts have been received about apparently diseased amphibians from a pet store and a private breeding facility. With follow-up investigations, no *Bsal* detections have yet been confirmed. *Bsal* sampling of animals at field sites in North America also have not resulted in *Bsal* detections at this time, either by HDAS or by more formal research and monitoring studies (e.g., Govindarajulu et al. 2017; Waddle et al. 2019, 2020; see Surveillance & Monitoring Working Group section below).

Relative to the fourth action, field mitigations in response to *Bsal* detections have had limited testing at this time. Garner et al. (2016) considered strategies to mitigate die-offs of wild amphibians from chytridiomycosis to be “at best, nascent”, with some approaches having ethical and legal considerations. Hopkins et al. (2018) identified regulations that were likely to be policy hurdles for habitat (e.g., chemical applications and ground disturbing activities with federal funding or on federal lands) and host (e.g., species of concern) management in response to conceptual *Bsal* die-off scenarios at United States field sites. In two different isolated instances of actual amphibian die-offs in Europe, field trials using a variety of actions were conducted to treat outbreaks of chytridiomycosis caused by *Bd* (Bosch et al. 2015) and *Bsal* (Martel et al. 2020). These actions included site biosecurity and habitat management as well as host removal, treatment, and surveillance, and some involved local to regional authorities for management action approval. Regulations aside, Bernard and Grant (2020) conducted a workshop to evaluate expert opinion on the efficacy of a variety of actions for *Bsal* mitigation at United States field sites. The uncertainties identified by the workshop highlight research needed to establish method efficacy within different species, habitat, and geographic contexts (see also Stegen et al. 2017). At the time of this writing, there is a recognized priority to clarify, improve, and aid navigation of North American policy regulations for *Bsal* management (see Management & Control Working Group section below) and to conduct research to advance understanding of the effectiveness of field mitigation actions (see Research Working Group section below).

Relevant to the fifth action, adding or heightening proactive biosecurity measures to forestall inadvertent human-mediated transmission of disease-causing pathogens is a topic that is gaining recognition and is highly relevant for *Bsal*. PARC’s national and regional Disease Task Teams have been aiding this effort: 1) within- and between-site biosecurity measures for amphibian field work to forestall pathogen transmission were discussed by Gray et al. (2017); 2) disinfection alternatives for large equipment used in or near amphibian habitats were

summarized by Julian et al. (2020); and, finally, 3) field-site contexts that could trigger use of enhanced biosecurity approaches rather than standard measures are currently being summarized. There is limited policy at the legislative level to enforce such biosecurity measures. Nevertheless, policies and practices are developed and observed among amphibian researchers at the institutional and agency levels. Specifically: 1) amphibian research permits through university or federal agency research programs' Institutional Animal Care and Use Committees (IACUCs) are increasingly addressing within- or between-field site biosecurity; 2) IACUC approvals require laboratory studies with *Bsal* to operate with heightened biosecurity levels, and standardized procedures for between-laboratory movement of *Bsal* cultures or samples have been established (see Research and Diagnostics Working Groups sections below); 3) some state and provincial scientific collection and use permits require language addressing disinfection protocols to safeguard amphibian health and welfare from human-mediated disease transmission; and 4) some research-reporting journals also require statements of biosecurity measures taken to forestall human-mediated spread of amphibian diseases, including *Bsal*. In addition, the World Organization for Wildlife Health (OIE 2020b) has discussed potential *Bsal* transmission by human-mediated movements of contaminated water and fomites. To date, no broad policies for clean water or fomite movements have been established. However, water treatment with chemicals for invasive species, including disease-causing pathogens such as *Bsal*, has been conducted during some water draws for regional wildfire management in the western United States (Olson et al. 2013, Olson and Ronnenberg 2014), and national guidance for such clean water transport has been established in the United States (NWCG 2017, 2020).

Despite this five-pronged approach to forestall *Bsal* emergence, which include various policies and standardized procedures, no single strategy or mix of strategies is failure-proof. Therefore, it remains imperative to continue developing and implementing proactive management to mitigate the risk of *Bsal* invasion while also pragmatically planning for the arrival of *Bsal* in North America. This approach is the foundational rationale for development of the North America *Bsal* Strategic Plan.



Fire Salamander (*Salamandra salamandra*). © Eric Isselée.

### III. History and structure of the North American *Bsal* Task Force

The *Bsal* Strategic Plan is a product of the North American *Bsal* Task Force. Its purpose is to outline steps to reduce the likelihood of *Bsal* transmission to North America and what is necessary for a successful response to the detection of *Bsal* in North America. A brief history of the *Bsal* Task Force and descriptions of the activities of the Technical Advisory Committee (TAC) and of each of the eight working groups follows.

#### **History of the North American *Bsal* Task Force**

At a 2015 workshop in Colorado, hosted by the US Geological Survey (USGS) Amphibian Research and Monitoring Initiative (ARMI) and the USGS Powell Center, researchers and managers discussed approaches to understanding the epidemiology of *Bsal* and its potential consequences on biodiversity losses in the Americas (Grant et al. 2015). The *Bsal* Task Force was conceived at this workshop (Fig. 2), and the Framework of Actions (Box 1) was developed to guide strategic planning along several themes, which are intended to be addressed simultaneously. Initially, seven interactive working groups (Fig. 2) were formed to address different goals that are integrated with the *Bsal* Task Force’s Framework of Actions: 1) Response; 2) Diagnostics; 3) Research; 4) Decision Science; 5) Surveillance & Monitoring; 6) Data Management; and 7) Outreach & Communication. Recently, the Response Working Group began focusing on mitigation actions in the event that *Bsal* is detected in North America, and this group has been renamed the Response & Control Working Group (Fig. 2). A Clean Trade Working Group was initiated in 2020 as the eighth working group. Progress of the *Bsal* Task Force and its component groups can be tracked by updates to the goals and priorities listed in the *Bsal* Implementation Plan (Appendix 5) and by activities listed in annual reports (available at [salamanderfungus.org](http://salamanderfungus.org)).

Since their formation, working groups have met via periodic conference calls to outline new tasks and discuss progress on existing efforts. Group membership is open and inclusive, but the groups were initially founded with persons involved with disease research, natural resource



management in state or provincial/territorial agencies and other government agencies, environmental or conservation groups, non-governmental organizations (NGOs), and the pet industry. Each group has one to three leads, who help to coordinate personnel, manage the workload, and serve as members of the TAC (Fig. 2).

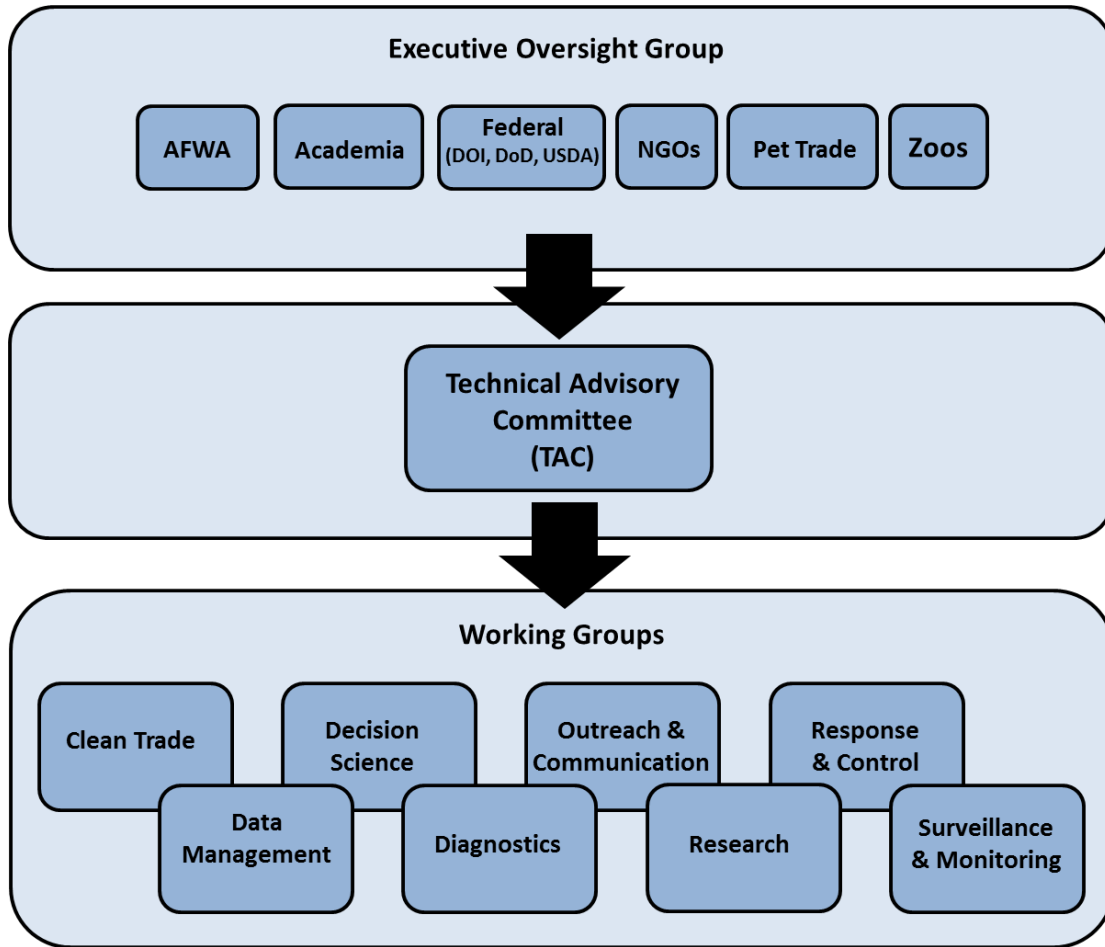


Figure 2. Structure of the North American *Bsal* Task Force.

### **Box 1. Framework of Actions**

Upon organization of the *Bsal* Task Force in 2015, nine guiding principles for strategic planning along several themes were developed to forestall *Bsal* emergence in North America. This Framework of Actions led to the development of eight working groups and associated collaborations among researchers, managers, industry, and other partners across North America.

1. Prevent invasion of *Bsal* into North America by encouraging stakeholders to work toward a clean trade program for amphibians that certifies individuals in trade are free of *Bsal* infection.
2. Develop and encourage use of the Rapid Response Plan (Appendix 4 in this Strategic Plan), which can be customized to meet local needs, to contain a *Bsal* outbreak.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of *Bsal* in animal or environmental samples in a timely manner.
4. Test for the occurrence of *Bsal* in Canada, Mexico, and the United States in the field and in captivity, reduce the risk of spillover from captive to wild amphibians, and reduce the likelihood of humans playing a role in the inadvertent translocation of *Bsal* within North America.
5. Advance the understanding of the risk of *Bsal* introduction to North America and assess the invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing *Bsal* surveillance data.
6. Develop effective, scientifically justified prevention and mitigation strategies that prevent *Bsal*-associated infections and mortality.
7. As evidence-based *Bsal* response and management actions are developed, identify expedited pathways for permitting actions and facilitate regulatory processes to implement mitigation measures.
8. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners that can communicate updates on *Bsal* developments and alert the public and scientific community if *Bsal* is detected in Canada, Mexico, or the United States.



Dusky Salamander (*Desmognathus fuscus*). © Alberto López.

#### IV. Technical Advisory Committee (TAC)

The Technical Advisory Committee (TAC) is populated by the working group leads; representatives from Canada, Mexico, and the United States; and representatives from partner groups, including different government agencies, the IUCN Amphibian Survival Alliance (ASA), the Pet Industry Joint Advisory Council (PIJAC), and Partners in Amphibian and Reptile Conservation (PARC). The roles of TAC members vary considerably, with strategic surveillance, research, and proactive planning being tri-lateral priorities across the three countries and advances in biosecurity procedures being contributed by PARC's national and regional Disease Task Teams. The TAC meets monthly by conference call to discuss ongoing work and allow for: 1) development or discussion of new information or tasks; 2) exploration of opportunities to collaborate on the development of products and grant proposals; and 3) discussion of communication, outreach, and networking needs.

**Objective:** To serve as an integrated leadership body of the North American *Bsal* Task Force in collaboration with key partners, framing the scope and intent of the activities undertaken by the *Bsal* Task Force as a whole and acting as an advisory panel for working groups and ad-hoc subgroups.

#### **Goals:**

**Goal 1.** Provide leadership of the *Bsal* Task Force and ensure progress toward the Framework of Actions.

**Goal 2.** Develop and strengthen lines of communication between the *Bsal* TAC, as representative of the entire *Bsal* Task Force, and national leaders to address risk and response to *Bsal* emergence in North America.

The *Bsal* Implementation Plan (Appendix 5) provides more specific priorities under each of these two goals. Goals and priorities may adapt over time. TAC accomplishments and activities, including adaptive management of goals and priorities, are provided in annual reports and periodic updates to the *Bsal* Implementation Plan (available at [salamanderfungus.org](http://salamanderfungus.org)).

The first goal of the TAC is designed to adapt over time to new knowledge discovery and activities of working groups and *Bsal* Task Force members and partners. The second goal of the TAC is intended to work toward development of a more formal network of relevant partners

among higher-level national leaders with jurisdiction over the natural heritage of species and biodiversity in Canada, Mexico, the United States, and the First Nations and Indigenous peoples of North America to serve as an Executive Oversight Group (Fig. 2 depicts this concept, but such a group is not formalized at this time). This oversight group was originally envisioned to be a mechanism to inform managers or leaders of new *Bsal* information or emerging *Bsal* topics at higher organizational levels, such as US federal agencies, the Association of Fish and Wildlife Agencies (AFWA), the Canadian Wildlife Service (CWS), the Mexican Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and PIJAC.

The initiation of a *Bsal* Task Force Executive Oversight Group was proposed to national leaders at the North American Wildlife and Natural Resources Conference in March 2016. Discussion there identified the need for such an oversight body to extend beyond *Bsal* and include other non-agricultural wildlife diseases with existing analogous task forces, such as white-nose syndrome in bats, as well as wildlife diseases without formalized task forces, such as sea star wasting disease. Such oversight for non-agricultural wildlife diseases is the topic of continued discussion and relates to a recognized gap in laws for wildlife health in the Canada, Mexico, and the United States. Although the US Animal Health Protection Act (7 USC § 109) covers agricultural wildlife health, there is no companion legislation for non-agricultural wildlife. Similarly, in Canada, the Health of Animals Act is targeted toward agricultural animal health, so the Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (WAPPRITA) is used to control the spread of *Bsal* via controlling salamander imports instead. These issues are examples of what the Executive Oversight Group could address.



Green Salamander (*Aneides aeneus*) infected with *Bsal*. © Todd Amacker.

## V. Working Groups

The eight working groups of the North American *Bsal* Task Force and their goals are introduced in this section. Additionally, ongoing working group tasks are described as priorities under each goal in the *Bsal* Implementation Plan (Appendix 5). The working groups are: Response & Control, Diagnostics, Research, Decision Science, Surveillance & Monitoring, Data Management, Outreach & Communication, and Clean Trade.

### 1. Response & Control Working Group

**Objective:** To provide guidance for the rapid response (including, but not limited to, eradication, containment, or other management and control responses) should *Bsal* be detected in North America.

**Background:** Natural resource managers are often faced with making rapid decisions. The process can be quite overwhelming and confusing when dealing with multiple factors, like an emerging pathogen, species with conservation status of concern, and differing state or provincial/territorial and federal jurisdictions, regulations, policy, and permitting. The *Bsal* Response & Control Working Group aims to bridge the gap between identifying and implementing scientifically-sound mitigation actions in response to a confirmed *Bsal* detection in North America by proactively designing guidelines, identifying permitting steps, and facilitating the process of navigating the requirements for state or provincial/territorial and federal policy. Simply put, the purpose of the group is to facilitate efficient and rapid response to *Bsal* invasion.

**Rapid Response Plan:** In 2015, an initial Response Working Group focused on development of a *Bsal* Rapid Response Plan Template, addressing Framework Action 2 (Box 1). After comprehensive review by federal, state, provincial, and other potential users of the Rapid Response Plan, this task has been completed, and the final product is included as Appendix 4

(see also [salamanderfungus.org](http://salamanderfungus.org)). The contents of the Rapid Response Plan are meant to be customized by any agency or institution with management jurisdiction over wild or captive salamanders so that the plan may serve as a template for actions required in case of suspected or confirmed *Bsal* detection.

The Rapid Response Plan Template is provided as an outline and guidance for local rapid response actions that could be triggered upon initial or subsequent detections of *Bsal*, in either wild or captive populations. The scenarios involve different levels of diagnostic information for sick or dead animals. In other words, all recommended actions occur after the laboratory has made its determinations based on the *Bsal* case definition (White et al. 2016). Proactive actions to forestall *Bsal* emergence are not considered in this Rapid Response Plan Template but are being considered by the Decision Science Working Group, which is developing guidelines for implementing management actions before an introduction or outbreak is suspected or confirmed. The Rapid Response Plan Template provides considerations for in situ containment (i.e., in the existing location of the population) as well as establishment of ex situ populations (i.e., outside of the natural location, such as in a captive assurance colony). Rapid containment and response measures may prevent broad impacts of the infection. The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact the ARMI Decision Science Lead, Dr. Evan Grant ([ehgrant@usgs.gov](mailto:ehgrant@usgs.gov)), for assistance. The Rapid Response Plan is considered a living document that will be updated as more information becomes available. Updates will be posted on relevant websites (e.g., [salamanderfungus.org](http://salamanderfungus.org)).

After completion of the Rapid Response Plan Template (Appendix 4) and development of scenario-building exercises (Hopkins et al. 2018), it became apparent that several aspects of responses to *Bsal* detection in North America warranted further development. These are tasks included within current goals (below) of the expanded Response & Control Working Group. Tasks and activities of the working group related to each goal are described further in the *Bsal* Implementation Plan (Appendix 5; [salamanderfungus.org](http://salamanderfungus.org)).

**Response & Control Working Group:** In 2018, the newly reconfigured Response & Control Working Group was formed to continue and expand the work described above. Specifically, the Response & Control Working Group aims to facilitate an efficient and effective response, by development of *Bsal* control mitigations, if *Bsal* were to be detected at a field or captive site in North America. This effort is part of Framework Actions 6 and 7 (Box 1), which also intersect the aims of other working groups, such as the Research and Decision Science Working Groups.

Mitigation strategies can target the host or environment (see also the Research Working Group section below). We can use what we have learned from *Bd* as a foundation for developing and understanding potential disease mitigation and treatment strategies and also take advantage of new, innovative ideas that are discovered through research. Host-directed strategies are mitigation tools aiming to foster disease resistance or tolerance, such as skin probiotics (Harris et al. 2009a,b; Bletz et al. 2013), vaccinations (McMahon et al. 2014, La Patra et al. 2015), and antifungal medications (Bosch et al. 2015, Hardy et al. 2015, Hudson et al. 2016). Environment-directed strategies include micropredator manipulations (Schmeller et al. 2014, Buck et al. 2011), salt augmentations (Stockwell et al. 2015a,b), environmental probiotics (Muletz et al. 2012), habitat alterations (e.g., fencing to reduce animal movements and human encroachment, water diversions to preempt *Bsal* transmission with water flow), and removal of infected hosts. These strategies have potential for mitigating *Bsal*'s impact on North American salamander biodiversity.

To implement *Bsal* management actions on the ground, government agencies may be required to follow national and/or state/provincial/territorial policies related to the potential environmental impacts resulting from such actions. For example, during scenario exercises of potential *Bsal* outbreaks in the United States (e.g., Hopkins et al. 2018), rapid responses were identified to be potentially slowed by lack of permits for United States federal or state authorities to assist in field responses including ground-disturbing activities, disturbance to species with conservation status of concern, and use of chemical applications to address the *Bsal* fungus viability. The Response & Control Working Group activities currently focus on understanding the permits and approvals that may be needed for various *Bsal* actions in field or captive settings and expediting rapid permit processes. An effort is underway to gain a better understanding of the complex network of permits and procedures in the United States and the processes that may expedite those approvals. Similar initiatives in Canada and Mexico are anticipated. Existing policies and procedures for the United States federal and state lands are described below.

**United States Federal Lands:** United States federal agencies are required to follow the policies of the National Environmental Policy Act (NEPA) of 1970. The NEPA process or “Environmental Impact Assessment process” applies when a federal agency has discretion to choose among one or more alternative means of accomplishing a particular goal. It requires agencies to determine if their proposed actions have significant environmental effects to land and water, protected wildlife and plants, historic properties, cultural resources, and other interests as well as to consider the environmental and related social and economic effects of their proposed actions. NEPA’s procedural requirements apply to a federal agency’s decisions for a variety of actions, including, but not limited to, permanent or temporary construction projects, limiting public access to public lands, using chemical or biological treatments, and permitting of private actions.

In the United States, private and state entities will often become involved in the NEPA process when applying for permits if they will be using public land access or public waters in their actions. The NEPA process is generally a long, drawn-out process that can be difficult to navigate if you are not familiar with it and can take years to accomplish, but the process must be completed before federal management decisions are made.

The Council on Environmental Quality (CEQ) oversees the NEPA process with the help of the Environmental Protection Agency (EPA), which issues permits for chemical and biologic use based on the Clean Water Act and Clean Air Act.

Once a proposed action has been developed, an agency can pursue one of two paths:

1. Environmental Assessment (EA), which determines the significance of the action’s effects and finds alternative measures.
2. Environmental Impact Statement (EIS), which must be prepared if an action significantly affects the quality of the human environment.

If an action may occur more than once, or it occurs routinely, and will not have a significant impact on the human environment (either positive or negative), the agency may seek a categorical exclusion (CE) from CEQ that precludes the need to prepare an EA or EIS for future actions, but the process for obtaining approval from CEQ for a CE is lengthy and complex. The need must be carefully justified, and CEs are rarely granted. However, on rare occasions, CEQ may exempt an action from NEPA under the following circumstances:

1. If the agency needs to take an action in response to an emergency, and the action would typically require preparation of an EIS, but there is insufficient time to follow the regular NEPA process, then the agency can work with CEQ to develop alternative arrangements for compliance with NEPA (40 CFR § 1506.11) and proceed immediately to mitigate harm to life, property, or important resources.
2. The NEPA analyses and document may involve classified information. If the entire action is classified, the agency will still comply with the analytical requirements of NEPA, but the information will not be released for public review. If only a portion of the information is classified, the agency will organize the classified material so that the unclassified portions can be made available for review (40 CFR § 1507.3(c)).

**United States State Lands:** There are currently 16 states with Environmental Quality Acts that require state and local agencies to perform EISs or at least Environmental Reviews (ER) before performing actions and applying for permits. Please see Appendix 3 for a list of states with Environmental Quality Acts, the specific act to which they are bound, and the governing body of the act.

**Response & Control Working Group Goals:**

**Goal 1.** Review and update the Rapid Response Plan Template as new information becomes available.

**Goal 2.** Facilitate and improve a natural resource agency's ability to take proactive and reactive actions to prevent occurrence and transmission of *Bsal* in North America.

**Goal 3.** Provide information and build understanding of *Bsal*, the *Bsal* Task Force, the Strategic Plan, and available management/mitigation options for federal agencies at the national and regional levels.

**Goal 4.** Brief and offer training to natural resource agencies (local to regional) about the North American *Bsal* Task Force and available management/mitigation options.

Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).



## 2. Diagnostics Working Group

**Objective:** To assist with the promotion of consistent standards among the wildlife health community for detecting *Bsal* and diagnosing *Bsal* chytridiomycosis; to serve as a forum for exchanging ideas, working out challenges, and providing consultation and expert advice concerning *Bsal* detection and chytridiomycosis diagnosis.

**Background:** Effective detection of novel disease-causing pathogens, such as *Bsal*, relies both on gross and microscopic (histopathologic) examination of lesions in affected animals and on the detection and correct identification of the pathogen through histochemical and molecular methods. Molecular detection of pathogens is a rapidly developing discipline largely reliant on technological advances in analyzing organismal DNA and RNA. Mortality due to *Bsal* was first described by Martel et al. (2013), who also provided a morphological description of the pathogen and the histopathologic lesions it caused and developed a polymerase chain reaction (PCR) assay specific to the 5.8S rRNA gene of *Bsal* that could be run on skin samples. As *Bsal* is closely related to *Batrachochytrium dendrobatidis* (*Bd*), and since bi-pathogen infections are possible, Blooi et al. (2013) developed a duplex real-time PCR assay to detect and differentiate both pathogens. Further advancing the development of reliable standardized diagnostic procedures for *Bsal* is an overarching goal for the working group.

The *Bsal* Diagnostics Working Group was convened as a partnership among diagnosticians and researchers interested in further development of effective diagnostics tools for *Bsal*, addressing Framework Action 3 (Box 1). The Diagnostics Working Group is composed of professionals with expertise in the application and interpretation of an array of diagnostic tools, with members working in academia, diagnostic laboratories, and government agencies throughout North America and involved in detection and reporting of amphibian diseases, including *Bsal*.

Since the working group's assembly in 2015, collaborations between members of the group and others have achieved several initial goals. First, the *Bsal* case definition (White et al. 2016) was published to establish criteria to promote standardized communication of diagnostic results for diagnosis of *Bsal*-caused disease, i.e., *Bsal* chytridiomycosis. The case definition describes the clinical and histopathological presentation of *Bsal* chytridiomycosis and references Martel et al. (2013) and Blooi et al. (2013) for molecular analyses by PCR. Since then, additional recommendations on diagnosing *Bsal* chytridiomycosis have been produced (see Thomas et al. 2018). A second achievement was the completion of a pilot multiple-laboratory round-robin proficiency test for *Bsal* detection by PCR in 2016 and the development of a methodology and logistics plan for a full round-robin, both with funding from Environment and Climate Change Canada. Third, an in situ hybridization protocol to detect *Bd* and *Bsal* cells in formalin-fixed paraffin-embedded tissues was developed (Ossiboff et al. 2019)—definitive differentiation of *Bd* and *Bsal* in tissue sections of affected amphibians is impossible based on fungal morphology and routine histologic stains alone. As the case definitions for *Bsal* and *Bd* chytridiomycosis require both histologic and molecular evidence of infection, this new test to simultaneously screen for and differentiate the two fungal pathogens in tissue sections is critical for accurate diagnosis. Ongoing work by the group is capitalizing upon the value of collaborations among diagnosticians and researchers with different expertise. Goals and priority actions per goal are

described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

**Diagnostics Working Group Goals:**

**Goal 1.** Establish a long-term program for inter-laboratory quality control and evaluation of protocols for the detection of wildlife pathogens, particularly *Bd* and *Bsal*.

**Goal 2.** Develop standardized and reproducible methods that will allow comparison across studies, with reliable detection of *Bd* and *Bsal* and reliable estimation of infection load once either or both pathogens are detected.

### 3. Research Working Group

**Objective:** To facilitate communication and collaboration among scientists studying *Bsal* in North America and to ensure high-quality research on *Bsal* is produced rapidly.

**Background:** The breadth of *Bsal* research needs span basic, applied, and theoretical science disciplines; advances in all three arenas are needed to build foundational knowledge essential for responding to *Bsal* emergence. Scientific studies across these arenas broadly address Framework Actions 4 through 6 (Box 1). To achieve a broad scope of work, the *Bsal* Research Working Group is a collaboration of scientists with a diversity of expertise from multiple disciplines, including molecular and cellular biology, immunology, ecology, mathematics, pathology, and social sciences. The group strives for inclusion and productive collaboration and to maintain or expand its participants to effectively address open research questions critical to our ability to respond to and manage *Bsal*. The working group is currently composed of >30 members, representing >20 organizations.

The Research Working Group maintains a list of research needs based on recent publications, our current state of knowledge, and key knowledge gaps. The working group ranks research needs on the list as Urgent, High, or Medium priority. Within each of these categories, research needs are considered to have equal importance. Each year, the list is updated as more information becomes available and ranks change. Ranks are associated with the thematic goals for the Research Working Group, listed below. The research goals identified below represent a comprehensive approach to advancing the understanding of *Bsal*'s potential impact on amphibian host communities and effective response and management approaches should *Bsal* be introduced into North America. The *Bsal* Implementation Plan (Appendix 5) further breaks down these goals into priority research studies (i.e., action items); achievements are listed in annual reports ([salamanderfungus.org](http://salamanderfungus.org)).

#### Research Working Group Goals:

**Goal 1:** Understand the role of human behavior and the pet trade in the spread and spillover of *Bsal*.

**Goal 2:** Identify critical transmission pathways and conditions under which *Bsal* is likely to emerge in amphibian host populations in North America (e.g., compartmental disease models).

**Goal 3:** Produce more informed *Bsal* risk models for North America through improved, objective classification of species susceptibility and tolerance to *Bsal* infection (e.g., integral projection models).

**Goal 4:** Identify effective methods for managing *Bsal*-induced disease and clearing *Bsal* infections in captive and field settings.

**Goal 5:** Quantify innate and adaptive immune responses to *Bsal* across species and environmental conditions.

**Goal 6:** Identify the mechanisms of *Bsal* pathogenesis.

**Goal 7:** Establish effective methods for detecting *Bsal* infections.

**Goal 8:** Estimate the interactive effects of *Bsal* with natural and anthropogenic stressors.

#### 4. Decision Science Working Group

**Objective:** To support management decisions regarding *Bsal* through the facilitation of decision-making processes, identification and collation of information needed to make decisions, development of models to predict the outcomes of different management options, and evaluation of trade-offs and risks to overcome impediments to optimal decision-making.

**Background:** The Decision Science Working Group applies the theory, tools, and techniques from decision analysis to the complex decision-making process for mitigating the threat of *Bsal*, managing risk to native amphibian communities, and responding to *Bsal* detections in North America (Framework Actions 2 and 5; see Box 1). Application of decision science provides an effective interface between rapidly changing conditions in societal, policy, and science information, uncertainty, and operational planning, which together provide substantial benefits that facilitate effective and rapid decision-making in response to *Bsal* detection. The Decision Science Working Group is currently composed of members representing academic institutions and federal agencies (US Geological Survey, US Fish and Wildlife Service). Collectively, the group has decades of experience in decision science, amphibian and pathogen ecology, research, mathematical modeling, and direct work with managers.

Emerging diseases have the potential to affect social, economic, and ecological interests of North American resource managers, who are entrusted by society to manage protected areas and wildlife populations. Because they must consider multiple objectives, resource managers face difficult trade-offs for any disease management strategy. The complexity that arises in balancing numerous, competing demands on resource managers effectively limits our ability to identify and implement proactive management, representing a major challenge for developing management strategies for *Bsal* and other emerging infectious diseases. To date, there are no viable treatment options available for *Bsal*, which limits the alternatives available for managers until effective treatments are identified (the Research Working Group has identified research priorities to address this knowledge gap). Much uncertainty remains, which also makes choosing an (untested) management action challenging. Decision science provides a framework for developing strategies and determining a course of action in the face of uncertainty. Additionally, even if treatments are identified, implementation may still be delayed if other management objectives are predicted to suffer; decision analysis helps identify optimal solutions across potentially competing management objectives.

Planning for the possible arrival of *Bsal* in North America illustrates several decision-making impediments common in the world of wildlife disease. First, despite calls for improved responses to emerging infectious diseases in wildlife, management is seldom considered until a disease has been detected in a population. Lack of resources (i.e., time, money, and personnel) are often cited as reasons for not taking pre-emptive actions, but reactive approaches often limit the potential for control and increase the total cost of a response. Second, while preventing the arrival of a pathogen is the most effective means of controlling emerging infectious disease, it is not fail-safe. Once present in a new area or population, emerging diseases have the potential to impact competing social, economic, and ecological interests of North American resource managers, and there are consequently difficult trade-offs for any given disease prevention or management strategy (e.g., optimal actions for managing a wildlife disease may result in declines in recreational or economic values). Finally, acting under high levels of uncertainty is a hallmark of wildlife disease management. Choosing an untested management action can be difficult for

managers to justify to the public, and acting in the face of uncertainty depends a great deal on an individual manager's tolerance for risk.

The application of decision science is increasing among natural resource agencies, as it provides rational and transparent frameworks for managing disease. Decision science tools, such as cost-benefit analysis or portfolio decision theory, can help managers better understand the opportunity costs of proactive action versus inaction. Multi-criteria decision analysis can be used to help examine trade-offs among competing social, political, economic, and ecological objectives. Finally, decision trees, expected value of information, and Bayesian belief networks are useful tools for understanding risk tolerance and examining the trade-offs between managing despite uncertainty and delaying action to gain additional information. By using the tools from decision science to facilitate conversations between researchers and wildlife managers and identify optimal management strategies, the Decision Science Working Group can help navigate the common pitfalls of developing and implementing proactive management solutions for *Bsal* ahead of an invasion and plan for thoughtful responsive management once *Bsal* arrives in a population.

Since 2015, the Decision Science Working Group has made considerable progress on its initial goals. The inaugural meeting that led to the development of the North American *Bsal* Task Force was originally planned as a *Bsal* decision science workshop sponsored by the US Geological Survey. That workshop led to a report that was one of the *Bsal* Task Force's initial accomplishments (Grant et al. 2015) and helped springboard the first product of the Decision Science Working Group (Grant et al. 2017), which addressed proactive measures to forestall *Bsal* emergence in North America. Simultaneously with the formation of the *Bsal* Task Force, initial efforts of an independent subgroup that became the heart of the Decision Science Working Group came to fruition as their United States risk assessment for *Bsal* emergence was published (Richgels et al. 2016). The Decision Science Working Group's current goals are designed to help researchers and managers identify and address the kinds of decision-making impediments outlined above and to advance the overall Framework Actions of the *Bsal* Task Force (Box 1). Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

### **Decision Science Working Group Goals:**

**Goal 1:** Identify critical research that has the highest value of information, which will lead to an improved ability to manage *Bsal*.

**Goal 2:** Identify approaches to improve proactive management of *Bsal* when risk or competing objectives are impediments to action.

**Goal 3:** Conduct and update *Bsal* risk assessments.

**Goal 4:** Frame *Bsal* management problems at regional and resource manager levels.

**Goal 5:** Identify whether management should consider proactive, reactive, or a combination of management strategies, dependent on the presumed presence and spatial distribution of *Bsal*.

## 5. Surveillance & Monitoring Working Group

**Objective:** To facilitate and coordinate the surveillance of *Bsal* in North America.

**Background:** A coordinated surveillance effort is aimed at detecting the initial introduction of *Bsal* into North America, thereby allowing for a more effective rapid response to forestall further transmission and helping to safeguard the native fauna of the continent (Framework Action 4; see Box 1). Management and conservation actions cannot proceed effectively without the fundamental information about when and where *Bsal* is introduced to North America. While opportunistic *Bsal* sampling improves the odds of detecting *Bsal* compared to not sampling at all (e.g., Muths et al. 2009), this haphazard approach is unlikely to detect *Bsal* at the onset of its invasion. Instead, the Surveillance & Monitoring Working Group aim is the early detection of *Bsal* to allow for an effective and rapid response, with the ultimate vision of conserving amphibian biodiversity.

Achieving a broad and robust surveillance network is difficult and expensive because of the labor involved. No single organizational entity has been identified that has this capacity. Instead, the emphasis has been on coordinating and encouraging sampling for *Bsal* by diverse partners such that something close to a reasonable level of surveillance is achieved. Initial efforts toward this goal are described below. However, because of the limitations of initial approaches, current efforts of the Surveillance & Monitoring Working Group are aimed at building an integrated network of partners in surveillance. This network will increase *Bsal* awareness, engage volunteer personnel and citizen scientists, utilize dispersed in-kind resources, and increase the amount of *Bsal* sampling.



**Figure 3.** Swabbing the skin of an Eastern Newt (*Notophthalmus viridescens*) to test for the presence of *Bsal*. Photo credit: Todd Amacker.

### Past Surveillance Efforts:

**United States:** In the United States, some sampling has been conducted by federal agencies. As part of the Surveillance & Monitoring Working Group’s primary objective, a one-time major sampling effort was conducted by the US Geological Survey Amphibian Research and

Monitoring Initiative (ARMI) in 2014 to 2017 (Waddle et al. 2019, 2020). This effort included sample sites across the United States, with resources allocated according to the estimated risk of *Bsal* occurrence as per the risk assessment model results developed by Richgels et al. (2016). Over 10,000 amphibians (mostly salamanders) were sampled. *Bsal* was not detected (Waddle et al. 2019, 2020; data archived at [amphibiandisease.org](http://amphibiandisease.org) by the Data Management Working Group, see below). ARMI continues to sample at a very low level in select areas where resources allow. In addition, the US Fish and Wildlife Service has done some sampling using their National Wild Fish Health Survey funding. There is an ongoing effort to sample in Appalachia, which is one of the high-risk areas (Richgels et al. 2016).

In the United States and elsewhere, independent science investigations into *Bsal* detections from skin swabs of selected species in specific geographies are ongoing. For example, four reports of joint *Bsal* and *Bd* sampling in North America were published in Herpetological Review in 2017 (Olson 2019).

In addition to sampling, in the United States, an iNaturalist website was set up as way to gather observations from the public of sick or dead amphibians that might need follow-up investigation. Similarly, the Partners for Amphibian and Reptile Conservation (PARC) national Disease Task Team set up a Herpetofaunal Disease Alert System (HDAS; email reports to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org); include photograph, species affected, location, other relevant episode information, observer's name; Gray et al. 2018) that provides another way to gather observations of sick or dead amphibians that might not otherwise be reported. At its start, communication occurred between iNaturalist and HDAS, although that connection has been dormant in recent years. Reports received via HDAS or, when available, through iNaturalist, are typically forwarded to the relevant state biologist, but in some cases, when deemed necessary, members of the Disease Task Team or state authorities can use their personal networks to help facilitate further investigation.

**Canada:** In Canada, the provincial and territorial governments are the lead jurisdiction for amphibian disease surveillance. The provinces of British Columbia (BC) and Ontario have conducted the most intensive *Bd* and *Bsal* monitoring programs to date. In Ontario, over 900 amphibians were sampled opportunistically along a latitudinal gradient over a four-year period (2014–2017). All samples were tested for *Bsal*, and all tests were negative (Christina Davy, Ministry of Natural Resources and Forestry, unpubl. data). In 2016, provincial biologists in BC sampled for *Bsal* within a small number of wild Rough-skinned Newt (*Taricha granulosa*) and captive (pet store) salamander populations on the south coast—one of the high-vulnerability zones identified by Yap et al. (2015). *Bsal* was not detected by quantitative polymerase chain reaction (qPCR) analyses in any swabs from the 82 wild newts and 15 captive salamanders sampled (Govindarajulu et al. 2017). In many provinces, including Alberta, Saskatchewan, Québec and Newfoundland, the current approach is one of passive surveillance in which *Bsal* investigations are triggered by unusual or mass amphibian mortality events. However, Ontario is considering low-level opportunistic sampling over the short-term, as resources allow. In addition, amphibians seized from the illegal pet trade will be tested for *Bsal* in BC.

The Canadian public can submit reports of sick or dead amphibians to the Canadian Wildlife Health Cooperative (CWHC). The CWHC is able to advise on the collection of carcasses for follow-up investigation and screens samples for diseases and parasites to assess the health of wild populations (CWHC 2019). Canadian provinces and territories may have additional reporting tools for sick or dead amphibians, such as the Government of British Columbia's



“Frogwatching” site, which is monitored by the provincial amphibian specialist. Disease reports from Canadian locations that are received by the HDAS email address are forwarded to provincial authorities in Canada, similar to state reporting of HDAS reports received in the United States.

**Mexico:** In Mexico, surveys aimed at identifying *Bsal* in natural populations have been conducted by members of Gabriela Parra-Olea’s research laboratory at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). So far, 119 individuals of 41 species (frogs and salamanders) have been sampled, and *Bsal* has not been detected by qPCR analyses in any of the swabs (Parra-Olea, unpubl. data). Additional surveys by Eria Rebollar (Centro de Ciencias Genómicas, UNAM) and Gabriela Parra-Olea in plethodontid salamanders and *Ambystoma* species across the Trans-Mexican Volcanic Belt were planned for 2020 but had to be postponed because of COVID-19 restrictions on travel and teaching. Parra-Olea and Rebollar have begun efforts to provide certification to both of these research laboratories so that legal amphibian imports in Mexico can be screened for *Bsal*.

Surveillance & Monitoring Working Group goals are listed below. Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group’s activities ([salamanderfungus.org](http://salamanderfungus.org)).

#### **Surveillance & Monitoring Working Group Goals:**

**Goal 1:** Facilitate and support a wide-reaching, ongoing, coordinated, and sustainable *Bsal* surveillance program in Canada, Mexico, and the United States.

**Goal 2:** Identify *Bsal* sampling efforts that are occurring outside of efforts coordinated by the *Bsal* Surveillance & Monitoring Working Group.

**Goal 3:** Support and facilitate sampling of amphibians in the pet trade.

**Goal 4:** Develop initial plans for post-detection monitoring if *Bsal* were to be detected at a field or captive site in North America.

## 6. Data Management Working Group

**Objective:** To develop an online *Bsal* and *Bd* data management portal to accelerate sharing of planned or completed surveillance projects and scientific studies to accelerate the pace of learning about these pathogens tied to emerging infectious diseases and foster integrated efforts in research and monitoring across North America and among global communities.

**Background:** The Data Management Working Group is a multi-partner collaboration to retain a *Bsal* and *Bd* database of world amphibian-chytrid occurrences and projects for co-production of knowledge to improve the science and management of these pathogens. In particular, this working group works closely with other groups in the *Bsal* Task Force, the University of California at Berkeley (UC Berkeley) and its online collaborative resource [AmphibiaWeb.org](http://AmphibiaWeb.org), and the US Forest Service, manager of the world *Bd* database through 2019, in previous collaboration with Imperial College, London (Olson et al. 2013, Olson and Ronnenberg 2014). Underscoring all sources of *Bsal* and *Bd* data are the independent investigators who have been vital to compiling and disseminating *Bsal* and *Bd* surveillance results. These data are now available via development of an accessible web portal database with data import and export, analysis, and multi-database linking functions (Framework Action 5 and 8; see Box 1). A web portal reporting system for *Bsal* and *Bd* data provides larger-scale data management capabilities and can address novel multi-scale questions and metadata analyses across individual studies. This data-sharing capability can contribute to analyses that advance understanding of the risk of *Bsal* or *Bd* introduction to North America and assess disease risk of these potentially deadly pathogens to native amphibians in North America and elsewhere through aggregating and managing past and current disease sampling data in a common repository.

The Data Management Working Group has met its original goal by developing the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)) to comprehensively manage data on *Bd* and *Bsal*. This standards-compliant, online portal is hosted as the Disease Portal webpage on [AmphibiaWeb.org](http://AmphibiaWeb.org), managed by UC Berkeley. The portal, which includes public and private datasets, is aimed at accelerating information sharing among global scientists, natural resource managers, and the public regarding planned and ongoing surveillance projects and scientific studies as well as the results of completed studies. Data within the portal are especially important for aiding in rapid responses and decision science for allocation of limited resources available for research and management of these amphibian emerging infectious diseases and their affected hosts. In particular, comprehensive management of *Bd* and *Bsal* detection and no-detection data by location and project is useful for development of new scientific research studies, surveillance, and effective monitoring programs and for understanding disease dynamics of chytridiomycosis. Through its website, in particular the Data Dashboard, the Amphibian Disease Portal could be an effective outreach and technical interface for the research community. Further, links to other online scientific portals, such as AmphibiaWeb, help extend the portal's reach to other audiences in education and conservation.

The Amphibian Disease Portal aims to expand in scope and capacity. In addition to increasing registered portal users and *Bsal* and *Bd* data imports, the portal is partnering with the global bio-sampling database Geome ([geome-db.org](http://geome-db.org)) to enhance research and forecasting abilities by: 1) delivering improved validation services, 2) enhancing security, and 3) improving data accessibility through third-party applications and programming tools (e.g., R statistical software and Python language applications). These developing functional capacity priorities of the web portal are described in the *Bsal* Implementation Plan (Appendix 5). Goals and priority

actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

**Data Management Working Group Goals:**

**Goal 1.** Maintain comprehensive data management of *Bd* and *Bsal* samples for archived, aggregated monitoring and analytic modeling in the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)).

**Goal 2:** Advance the functional capacity of the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)).

## 7. Outreach & Communication Working Group

**Objective:** To facilitate *Bsal* communication and outreach in North America.

**Background:** The Outreach & Communication Working Group produces a variety of *Bsal*-related outreach communication materials, including a web presence ([salamanderfungus.org](http://salamanderfungus.org)), fact sheets, press releases, lay and scientific articles, briefing papers, blog posts, and social media posts (Facebook and Twitter). In particular, the Outreach & Communication Working Group works with the Technical Advisory Committee (TAC) and its partners and other working groups within the *Bsal* Task Force to disseminate new *Bsal* information and products developed by the group and others. To increase the efficacy of dissemination, the Outreach & Communication Working Group continues to build an online network via social media, increasing followers and directing them to the [salamanderfungus.org](http://salamanderfungus.org) website, which serves as a hub and repository for published developments relating to issues, detections, and research regarding *Bsal*.

Although the Outreach & Communication Working Group is not tasked with conducting or publishing *Bsal* research, members help synthesize findings and communications from other groups within the *Bsal* Task Force for the purposes of producing lay articles meant to educate the public and highlighting key messages in social media posts. The scope of outreach to date has focused on national coverage in the United States, as there are salamanders at risk throughout the United States. Outreach may also be relevant for Canada and Mexico, but focused efforts outside the United States have not yet been initiated by the working group.

Finally, the Outreach & Communication Working Group organizes, designs, and publishes the annual report for the *Bsal* Task Force. This annual report summarizes advancement within all working groups and the current status of the *Bsal* fungus. This report is published on [salamanderfungus.org](http://salamanderfungus.org) and is available to both the general public and the scientific community. Goals of the working group are listed below, and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5; [salamanderfungus.org](http://salamanderfungus.org)).

### Outreach & Communication Working Group Goals

**Goal 1.** Work with partners to disseminate syntheses, research, and other products or activities developed by the *Bsal* Task Force via social media and newsletter articles.

**Goal 2.** Continue to build a network on social media to communicate developments within the *Bsal* Task Force.

**Goal 3.** Independently, or with partners, produce public service announcements on the presence and implications of *Bsal*.

**Goal 4.** Update the *Bsal* Task Force website.

## 8. Clean Trade Working Group

**Objective:** To expand efforts to forestall potential human-mediated transmission of *Bsal* into North America via trade markets.

**Background:** Although *Bsal* was first described from infections of Fire Salamanders in Europe, an Asian origin of the pathogen was implicated from the outset (Martel et al. 2013). Additional support for the hypothesis that *Bsal* evolved in Asia has been forthcoming. No lethal infections have been found there, suggesting that a long co-evolutionary history has led to resistance or tolerance by amphibian species in Asia (Laking et al. 2017). In Europe, infections were first observed in the Netherlands (Spitzen-van der Sluijs et al. 2016), followed by the discovery of *Bsal* in Belgium and Germany (Stegen et al. 2017, Lötters et al. 2020) and most recently in Spain (Martel et al. 2020). The lethal effect of *Bsal* on some European amphibian species suggests a recently arrived pathogen that has encountered naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations; possibly movement of spores by waterfowl, other wildlife, and humans; and spillover of infected individuals once held in captivity (Nguyen et al. 2017, Yuan et al. 2018). Spread of *Bsal* between continents, including the emergence of *Bsal* in Europe, is likely due to importation of infected species from locations where *Bsal* is endemic (Nguyen et al. 2017). Anurans from Asia infected with *Bsal* have been found in a pet store in Germany (Nguyen et al. 2017, Yuan et al. 2018). In addition, *Bsal* has been found on salamander species in China that are frequently imported. These findings suggest a role of trade markets in the between-continent spread of *Bsal*. In particular, the discovery that anurans can be infected opens up the possibility that trade in frogs for food, research, and pets can lead to between-continent dispersal. Clean trade measures, such as development of *Bsal* testing and certification procedures that help ensure traded animals and fomites in imports to North America are *Bsal*-free, are likely the most effective proactive measures for forestalling *Bsal* emergence in North America (Grant et al. 2017). Such measures align with Framework Action 1 (Box 1).

The Clean Trade Working Group was established in early 2020 in partnership with the Pet Industry Joint Advisory Council (PIJAC), and its implementation goals and priorities are under development at this time. The objective of the stakeholder-led Clean Trade Working Group is for experts in the pet trade to organize and collaborate with subject matter experts, regulators, and academics to determine what the potential components of a workable, comprehensive clean trade program for North America may include and to report these initial findings back to the *Bsal* Task Force Technical Advisory Committee (TAC). Currently, this working group has established a network of experts in amphibian and reptile care, husbandry, and pathogen and disease research, including industry experts with knowledge of the volume and dynamics of the amphibian trade. This group has been collaborating on a regular basis to identify potential components for a comprehensive North American clean trade program to help prevent the invasion of *Bsal* to North America while allowing for the legal and responsible importation of animals for the pet trade. For example, components under discussion at this time include a description of the current status of amphibian imports; a characterization of the United States and Canada amphibian trade network; and identification of factors or processes that could reduce potential pathogen transmission, amplification, and spillover.

## Appendices



Three-lined Salamander (*Eurycea guttolineata*). © Alberto López.

## Appendix 1. Results of *Batrachochytrium salamandrivorans* (Bsal) Susceptibility Trials

Table A1.1. The summarized results presented below were obtained from Martel et al. 2014 as well as unpublished data provided by M. Gray (University of Tennessee), D. Woodhams (University of Massachusetts), and J. Piovia-Scott (Washington State University).

### Species that developed *Bsal* chytridiomycosis (i.e., lethal and susceptible species)

Common Name	Scientific Name	Family
Dusky Salamander	<i>Desmognathus auriculatus</i>	Plethodontidae
Red Salamander	<i>Pseudotriton ruber</i>	Plethodontidae
Blue Ridge Two-lined Salamander	<i>Eurycea wilderae</i>	Plethodontidae
--	<i>Aquiloerycea cephalica</i>	Plethodontidae
--	<i>Chiropterotriton</i> spp.	Plethodontidae
Eastern Newt	<i>Notophthalmus viridescens</i>	Salamandridae
Striped Newt	<i>N. perstriatus</i>	Salamandridae
Black-spotted Newt	<i>N. meridionalis</i>	Salamandridae
Rough-skinned Newt	<i>Taricha granulosa</i>	Salamandridae

### Species that are *Bsal* carriers (i.e., tolerant species)

Marbled Salamander	<i>Ambystoma opacum</i>	Ambystomatidae
Blue-spotted Salamander	<i>A. laterale</i>	Ambystomatidae
Mexican Axolotl	<i>A. mexicanum</i>	Ambystomatidae
Ocoee Salamander	<i>Desmognathus ocoee</i>	Plethodontidae
Seepage Salamander	<i>D. aeneus</i>	Plethodontidae
Southern Two-lined Salamander	<i>Eurycea cirrigera</i>	Plethodontidae
Southern Gray-cheeked Salamander	<i>Plethodon metcalfi</i>	Plethodontidae
Eastern Red-backed Salamander	<i>P. cinereus</i>	Plethodontidae
Eastern Hellbender	<i>Cryptobranchus alleganiensis</i>	Cryptobranchidae
American Toad	<i>Anaxyrus americanus</i>	Bufoidea
Chiricahua Leopard Frog	<i>Lithobates chiricahuensis</i>	Ranidae
Cope's Gray Treefrog	<i>Hyla chrysoscelis</i>	Hylidae
Eastern Spadefoot	<i>Scaphiopus holbrooki</i>	Scaphiopodidae

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**Species that are resistant to *Bsal***

Spotty Dusky Salamander	<i>Desmognathus conanti</i>	Plethodontidae
Seal Salamander	<i>D. monticola</i>	Plethodontidae
Red-legged Salamander	<i>Plethodon shermani</i> x <i>teyahalee</i>	Plethodontidae
Four-toed Salamander	<i>Hemidactylium scutatum</i>	Plethodontidae
Cave Salamander	<i>Eurycea lucifuga</i>	Plethodontidae
Mudpuppy	<i>Necturus maculosus</i>	Cryptobranchidae
Wood Frog	<i>Lithobates sylvaticus</i>	Ranidae



## Appendix 2. Records of *Batrachochytrium salamandrivorans* (Bsal) in Nature and in Captivity.

Table A2.1. Summarized records of species infected with *Batrachochytrium salamandrivorans* (Bsal) in wild and captive settings.

Common Name	Scientific Name	Family	Location	Disposition (Wild/Captive)	Citation
Chinese Giant Salamander	<i>Andrias davidanus</i>	Cryptobranchidae	China	Captive	Yuan et al. 2018
Many-webbed Fire-bellied Toad	<i>Bombina microdeladigitora</i>	Bombinatoridae	Germany	Captive	Nguyen et al. 2017
Bosca's Newt [Iberian Newt]	<i>Lissotriton boscai</i>	Salamandridae	UK	Captive	Fitzpatrick et al. 2018
Anatolia Newt [Strauch's Spotted Newt]	<i>Neurergus strauchii</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
Eastern Newt	<i>Notophthalmus viridescens</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
Macedonian Crested Newt	<i>Triturus macedonicus</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
North African Fire Salamander	<i>Salamandra algira</i>	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Vietnamese Crocodile Newt	<i>Tylototriton vietnamensis</i>	Salamandridae	Vietnam	Captive	Martel et al. 2014
Many-webbed Fire-bellied Toad	<i>Bombina microdeladigitora</i>	Bombinatoridae	Vietnam	Wild	Nguyen et al. 2017
Chuxiong Fire-bellied Newt	<i>Cynops cyanurus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Chinese Fire-bellied Newt	<i>Cynops orientalis</i>	Salamandridae	China	Wild	Yuan et al. 2018
Dayang Newt	<i>Cynops orphicus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Clouded Salamander	<i>Hynobius nebulosus</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Netherlands	Wild	Martel et al. 2014
Smooth Newt	<i>Lissotriton vulgaris</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Japanese Clawed Salamander	<i>Onychodactylus japonicus</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
--	<i>Pachytriton wuguanfui</i>	Salamandridae	China	Wild	Yuan et al. 2018
Asian Warty Newt sp.	<i>Paramesotriton aurantius</i>	Salamandridae	China	Wild	Yuan et al. 2018
Hong Kong Warty Newt	<i>Paramesotriton hongkongensis</i>	Salamandridae	China	Wild	Yuan et al. 2018
Tam Dao Salamander	<i>Paramesotriton deloustali</i>	Salamandridae	Vietnam	Wild	Martel et al. 2014
Tam Dao Salamander	<i>Paramesotriton deloustali</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017

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Common Name	Scientific Name	Family	Location	Disposition (Wild/ Captive)	Citation
--	<i>Paramesotriton</i> sp.	Salamandridae	Vietnam	Wild	Laking et al. 2017
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Germany	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Martel et al. 2013
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Netherlands	Wild	Martel et al. 2014
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Martel et al. 2014
Siberian Salamander	<i>Salamandrella keyserlingii</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
Black Knobby Newt	<i>Tylototriton asperrimus</i>	Salamandridae	China	Wild	Laking et al. 2017
Black Knobby Newt	<i>Tylototriton asperrimus</i>	Salamandridae	Vietnam	Wild	Yuan et al. 2018
Chiang Mai Crocodile Newt	<i>Tylototriton uyenoii</i>	Salamandridae	Thailand	Wild	Martel et al. 2014
Himalayan Newt	<i>Tylototriton verrucosus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Vietnamese Crocodile Newt	<i>Tylototriton vietnamensis</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
Ziegler's Crocodile Newt	<i>Tylototriton zieglerei</i>	Salamandridae	Vietnam	Wild	Martel et al. 2014
Ziegler's Crocodile Newt	<i>Tylototriton zieglerei</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
Sword-tailed Newt	<i>Cynops ensicauda</i>	Salamandridae	--	Wild*	Martel et al. 2014

## Appendix 3. List of US States with Environmental Quality Acts and List of Provincial Amphibian–Reptile Specialists in Canada

Table A3.1. List of US states with Environmental Quality Acts and the governing body to contact for information. EIS = Environmental Impact Statement. EA = Environmental Assessment. ER = Environmental Review. Information available at: <https://ceq.doe.gov/laws-regulations/states.html>. See also: Council on Environmental Quality, A Citizen’s Guide to the NEPA, 2007: [https://ceq.doe.gov/docs/get-involved/Citizens\\_Guide\\_Dec07.pdf](https://ceq.doe.gov/docs/get-involved/Citizens_Guide_Dec07.pdf).

State	Governing Act	Governing Body	Notes
California	California Environmental Quality Act (CEQA) 1970	Attorney General	Require EIS for Local Projects, requires ER for individual businesses for agriculture, requires statements for potential impact on Climate change
Connecticut	Connecticut Environmental Policy Act (CEPA) 1973		
Georgia	Georgia Environmental Policy Act (GEPA)1991		Require EIS for local governments If the cost is greater than \$250k or the state pays for more than 50% of action
Hawaii	Hawaii Environmental Policy Act (HEPA) 1974	Hawaii Office of Environmental Quality (OECQ)	
Indiana	Indiana Environmental Policy Act (IEPA) 1972	Indiana Department of Emergency Management	
Maryland	Maryland Environmental Policy Act (MEPA) 1973	Maryland State Legislature	Only required when it’s a legislative action (gov pays for it)
Massachusetts	Massachusetts Environmental Policy Act 1977		
Minnesota	Minnesota Environmental Policy Act 1973		Requires EIS for local governments, requires ER for agriculture actions
Montana	Monatan Environmental Policy Act 1971	Montana Environmental Quality Council	
New Jersey	Executive Order 215 (1989)	New Jersey Department of Environmental Protection	
New York	NY State Environmental Quality Review Act (SEQR) 1978	State and Local Government	Require EIS for local governments. Requires ER for agriculture, State and Local gov permits are required. Citizens may sue the state if an action if “harmed” by an action. Require an EIS for climate change
North Carolina	North Carolina Environmental Policy Act 1971	North Carolina Department of Environmental and Natural resources	
South Dakota	South Dakota Environmental Policy Act 1974		
Virginia	Virginia Code Sections 10.1-10.1188 (1973)	Virginia Department of Environmental Quality and other state agencies	More than one agency may b required to permit depending on the action
Washington	Washington Environmental Policy Act (SEPA) 1971	Washington Department of Environmental Quality	Require EIS for local governments
Wisconsin	Wisconsin Environmental Policy Act (WEPA) 1974	State Controller and Wisconsin Department of Natural Resources	

Table A3.2. List of provincial amphibian–reptile specialists in Canada.

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## Bsal Strategic Plan

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## Appendix 4. Rapid Response Template

**Last revised January 28, 2019**

**PLEASE NOTE:** Within this document are explanatory notes and questions to stimulate discussion to help clarify the intent of the information provided for end users and to facilitate their customization of the template. These notes are placed throughout the text in [bracketed blue font] to distinguish them from other guidance provided for the purpose responding to a detection or outbreak of *Bsal*.

**Purpose:** This document is a **template to be customized** by any agency or institution with management jurisdiction over wild or captive salamanders, respectively, when actions in response to a disease may be warranted. [This purpose statement may be further customized as needed for individual entities.]

Herein are provided an outline and guidance for **local, rapid response** actions that could be triggered upon initial or subsequent detections of *Bsal*, in either wild or captive populations. [The scenarios are based on what an entity using this plan might do after receiving information regarding *Bsal* detection status from a diagnostic laboratory with expertise in *Bsal* diagnostics. In other words, all recommended actions occur after the laboratory has made its determinations based on the case definition of *Bsal* (White et al. 2016).] Also provided are considerations for in situ containment (i.e., in the existing location of the population) as well as establishment of ex situ populations (i.e., outside of the natural location, such as in a captive assurance colony). Rapid containment and response measures may prevent broad impacts of *Bsal*. [The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact the ARMI Decision Science Lead, Dr. Evan Grant ([ehgrant@usgs.gov](mailto:ehgrant@usgs.gov)), for assistance.]

This template was produced by the *Bsal* Response Working Group as part of their work with the *Bsal* Task Force's Technical Advisory Committee (TAC) (see [www.salamanderfungus.org](http://www.salamanderfungus.org) for additional information) and is considered a living document that will be updated as more information becomes available. At the time of this version of the template, *Bsal* is not known to occur in North America, and suggested responses are consistent with the high-alert condition of *Bsal* being yet undetected or rare in North America.

This document is intended to be incorporated into a National *Bsal* Strategic Framework, where larger surveillance and monitoring strategies, research needs, policy needs, and related prevention strategies, along with public outreach and communication, are addressed.



*Batrachochytrium salamandrivorans* (*Bsal*)  
Rapid Response **Template**

**Preliminary definitions and resources:**

This section sets forth how terms are defined within the rest of the document. [\[Entities customizing this template should add other definitions as they deem appropriate.\]](#)

**Definitions:**

- 1) ***Bsal*-susceptible host species** – We use this phrase broadly to indicate both species for which *Bsal* can be fatal and species that can be infected by *Bsal* but not develop signs of disease, thereby serving as carriers and reservoirs of *Bsal*. At the time of this writing, experimental evidence suggests that anurans may carry *Bsal* (Stegen et al. 2017). Thus, we assume that all amphibian species *may* be susceptible to *Bsal* or be carriers of *Bsal* unless it is demonstrated that a species cannot be infected. [\[\*Bsal\* has been termed the “salamander fungus” because it was described from infected Fire Salamanders \(\*Salamandra salamandra\*\) in Europe and has been shown to either infect or to be carried by several other salamander species \(Martel et al. 2014\). New evidence suggests that some anurans can also be infected and carry the pathogen, potentially without developing clinical signs of infection \(Stegen et al. 2017, Yuan et al. 2018\). This template and this definition will be updated when new evidence of species-specific susceptibility becomes available.\]](#)
- 2) **Wild host population** – Free-ranging population of *Bsal*-susceptible species.
  - a. Naïve (no prior *Bsal* detections known at a given site)
  - b. Exposed (prior *Bsal* detections documented at a given site)
  - c. Unknown (no or insufficient *Bsal* surveillance has been performed)
- 3) **Captive host population** – Any population that is not free-ranging, including outdoor enclosed spaces or fenced runs where contact with wild amphibians or disease vectors may be possible (e.g., zoo, aquarium, research facility, university).
  - a. Naïve (no prior *Bsal* detections known from the captive location)
  - b. Exposed (prior *Bsal* detections documented from the captive location)
  - c. Unknown (no or insufficient *Bsal* surveillance has been performed)
- 4) **Mortality event, wild** – Death of one or more free-ranging amphibians in the environment, whether or not the *Bsal* pathogen has been detected.
- 5) **Mortality event, captive** – Death of one or more amphibians in a captive environment, whether or not the *Bsal* pathogen has been detected.

- 6) **Eradication** – The assumed elimination of *Bsal* from individual amphibians *in captivity* based on four consecutive negative PCR tests, each one week apart, per individual, as described in Blooi et al. (2015a).
- 7) **Participating Laboratory** – The particular laboratory that has been engaged during a testing or response effort; see also the Resources section below regarding the Diagnostic Laboratory Network.
- 8) **Reporting Individual(s)** – The individual(s) who submitted the sample(s) (e.g., swabs, carcasses, live animals) to a laboratory for diagnostics. This is the person(s) the laboratory is to contact to provide results. In some cases, this person may be a scientific researcher. [At the time of this version, *the Bsal Task Force is developing a statement and working with key scientific journal editors to ensure that sharing of scientific findings with management agencies to facilitate early detection and rapid response actions will not diminish the value or integrity of the scientific findings or the person(s) involved.*]
- 9) **Core Response Team (CRT)** – The group of authorized professionals, and other parties involved in the initial discovery, that evaluates the situation and makes recommendations for next steps. The CRT may include other trusted parties, as appropriate, where information can be securely shared and will not compromise scientific integrity (see suggested CRT composition in Resources). [We reference the use of such a team as part of the recommended actions in the response scenarios described in this template. We suggest that certain members of this team be identified in advance to facilitate a rapid response. Below, we offer additional suggestions regarding the team’s role and composition. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.]

## **Resources**

- A. **Diagnostic Laboratory Network.** A consortium of participating laboratories equipped to handle *Bsal* testing requests and to employ specific protocols (as recommended by the *Bsal* Task Force’s Diagnostics Working Group) for quality assurance and quality control (QA/QC). The network assists with coordination of sample handling. The list of known laboratories capable of *Bsal* testing is provided on the *Bsal* Task Force website ([www.salamanderfungus.org/resources/labs](http://www.salamanderfungus.org/resources/labs)). [Entities customizing this template may benefit from contacting their nearest laboratory(-ies) to understand their sample submission protocols, fees for services (as applicable), and any other requirements relevant for sample submission in the event of a disease outbreak (whether *Bsal* or another pathogen).]
- B. **Core Response Team (CRT).** [As noted above, we reference the use of such a team as part of the recommended actions in the response scenarios described in this template.]

Here, we offer suggestions on the charge and composition of the team. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.]

1. **Purpose:** The CRT is an advisory group that discusses the specific response scenario and helps to make initial decisions regarding response actions and related communications. Any member of the CRT is expected to keep the shared information **confidential** until the **management agency or entity with jurisdiction** (i.e., the authority to make decisions about the species or the lands affected) indicates how, where, and when information may be shared.
2. **Composition:** [The composition of this team may change depending on the specific circumstances. Below is a brief explanation of the suggested composition. Note that the ***Bsal* Technical Advisory Committee** includes appropriate expertise in the event of a *Bsal* outbreak and is at your disposal for confidential advisory assistance.]
  - i. Reporting Individual(s) – [The individual who discovered the mortality event or was involved in research that led to a *Bsal*-positive detection may have ability to assist in response-related actions or follow-up work at the site.]
  - ii. Agency or entity with jurisdiction over the affected species or lands – [The agency with management jurisdiction or the land manager will be able to confirm actions that can or cannot be taken.]
  - iii. Land or facility manager(s)/owner(s) where samples were collected, if different from the entity in (2)(b)(ii)
  - iv. State agency personnel in charge of amphibians – [The state or provincial/territorial fish and wildlife agency is the primary management authority for amphibians and can assist with appropriate species management actions on non-federal lands.]
    1. NOTE: The Association of Fish and Wildlife Agencies’ Amphibian & Reptile Program Manager can assist in determining the appropriate state contacts.
  - v. Key amphibian expert scientists who can provide recommendations, **in a confidential consulting capacity**, for short and long-term responses based on best available science – [Amphibian experts can advise on most current science.]
    1. NOTE: Please consider contacting the *Bsal* Technical Advisory Committee leadership ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)); one or

more members will be available to assist in a confidential advisory capacity.

**C. Points of contact (PoCs).** [Entities customizing this template should populate it with the appropriate PoCs.]

1. Compile a list of key contacts in a given state, federal agency, or management unit (e.g., unit director or manager, staff veterinarian, lead herpetologist, or wildlife biologist) to inform and coordinate response actions to a positive diagnostic test result.
2. Include permit coordination contacts (state, federal, provincial, local, etc.)

**D. Wildlife Health Expert Networks.** Qualified wildlife experts to assist in treatment of captive or privately-owned animals and in issuing health certifications or other documentation to verify animal health, emergency responses, etc. may be found via:

1. The Diagnostic Laboratory Network established via the *Bsal* Task Force's Diagnostics Working Group (see [www.salamanderfungus.org](http://www.salamanderfungus.org)).
2. Veterinary experts:
  - i. [Association of Reptile and Amphibian Veterinarians](#) (ARAV)
  - ii. [American College of Zoological Medicine](#) (ACZM) list of board-certified zoological medicine veterinarians
  - iii. The [American Association of Wildlife Veterinarians](#) (AAWV)
  - iv. The [Canadian Wildlife Health Cooperative](#) (CWHC)
3. Wildlife epidemiologists or wildlife disease ecologists

**E. Facilities.** A list of available captive housing or breeding facilities (e.g., [Amphibian Ark](#) [AArk], [Canada's Accredited Zoos and Aquariums](#) (CAZA), [Association of Zoos and Aquariums](#) [AZA]-accredited zoos, or other local facilities), with contacts.

1. Treatment. [Entities customizing this template should identify secure emergency facilities in their network to temporarily house moribund (dying, unable to right themselves) or sick but potentially treatable animals.]
2. Rescue colonies. [Entities customizing this template should identify facilities to house rescued animals or those collected for the purpose of captive breeding and reintroduction.]
3. Museums or other storage facilities. [Entities customizing this template should identify facilities for vouchered animals or archived tissue samples, swabs, or extracted DNA.]

### Questions

What AArk, CAZA, or AZA facilities are local? Are you familiar with the appropriate contacts there? What local museums are able to accession animals? Can they also accession tissues, swabs, DNA?

- F. Protocols.** [Along with those below, consider other protocols that may be useful, e.g., data submission or management protocols.] Recommended guidance can be found at the Bsal Task Force website ([www.salamanderfungus.org](http://www.salamanderfungus.org)) via the Diagnostics or Research pages but see also Pessier and Mendelson (2017), including:
- i. Biosecurity protocols for field, lab, use of live cultures, etc.
  - ii. Swabbing and storage (and transportation) protocols.
  - iii. **See also Appendix I**, where pertinent portions of the guidance manual have been included and adapted for quick reference.

## SCENARIO-SPECIFIC MANAGEMENT RESPONSES

**NOTE:** The scenarios below pertain to mortality or PCR detection events (and subsequent confirmation of causative agent); however, any suspicious-appearing amphibians should be investigated. Examples of suspicious-appearing amphibians that should be reported include sick or lethargic individuals, those with black circular or oblong lesions, or those unable to right themselves. The Partners in Amphibian and Reptile Conservation (PARC) national Disease Task Team has established the “Herpetofaunal Disease Alert System” (HDAS) to help connect people to the appropriate experts and authorities quickly; please see the [PARC Disease Task Team website](#) for information and for how to send a report to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org).

### Scenario 1: Mortality event, cause unknown; **Wild**

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These actions should be implemented at the discretion of the jurisdictional management unit depending on the level of response they are able to take to help minimize potential impacts. Contact [\[your local amphibian expert or member of a Veterinary Expert Network\]](#) to assist. [\[Entities customizing this document should identify appropriate amphibian experts local to their jurisdiction.\]](#)

**When uncertain how to proceed or whom to contact,** the PARC Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org).

### Mandatory action:

**Notification to agency with management jurisdiction.** To facilitate early *Bsal* detection and rapid response, contact the management agency with jurisdiction where the mortality event occurred (which may be your own agency) to ensure they are aware of the testing event and impending results. [\[Important – Given the heightened state of alert for \*Bsal\* and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the management agency with jurisdiction where the mortality event occurred, even if this may be your own agency, to be sure they are aware that a mortality event has occurred and testing is underway, while results are pending.\]](#)

### Questions

Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state? (If not, the PARC Disease Task Team may be able to assist; send a message to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org) requesting information on the appropriate contacts.) **For management agencies:** Are there other partners that you need to engage, and if so, should it be at this stage or after results are received?

**Recommended actions (in no priority order and as feasible):****1) Tissue collection for diagnostics.**

- a. Collect any live but apparently moribund (dying, unable to right themselves) or lethargic animals, using humane euthanasia procedures, as applicable (see Appendix I, Section A), for submission to Participating Laboratory. Swabs alone are insufficient to confirm a *Bsal* diagnosis.
  - b. Collect carcasses, fresh-dead (see Appendix I, Section A), for diagnostic necropsy and submission to Participating Laboratory.
  - c. Sample other live amphibians (e.g., swabbing skin for use in a PCR assay), if area is high risk and if feasible (Appendix I, Section B).
- 2) Biosecurity protocols**, as established (Appendix I, Section A(3)), implemented for all field gear, especially as part of implementing action #2 above, and also upon leaving the die-off site.

**Question**

Have you considered establishing an approved set of biosecurity protocols for sampling or surveillance in a disease-affected site?

**3) Heightened alert considerations.**

- a. Increased surveillance
  - b. Local personnel notification. [It may be helpful to form and consult the CRT (see [Resources](#) above) or to assess notifications at this stage; notifications could be handled on a “need to know” basis.]
- 4) Containment considerations.** The following are options that might help prevent spread of pathogens.
- a. Restricted public access to the die-off site.
  - b. Signage at or around the exposed area(s).
  - c. Local personnel notification and access restrictions to the exposed area(s). [Again, it may be helpful to consult the CRT or to assess notifications at this stage, and notifications could be handled on a “need to know” basis.]
- 5) See “Definitive detection, **Wild**” scenario below for additional responses.**

## Scenario 2: Mortality event, cause unknown; **Captive**

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These actions should be implemented at the discretion of the captive management facility depending on how conservative or comprehensive of a response they are able to take to help minimize impacts. Contact [your local amphibian expert or member of a [Veterinary Expert Network](#)] to assist.

**When uncertain how to proceed or whom to contact**, the Partners in Amphibian and Reptile Conservation (PARC) Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org).

### **Mandatory action:**

**Notification to state or provincial/territorial fish and wildlife agency.** To maintain transparency and open communications regarding *Bsal* and to facilitate early detection and rapid response, we recommend contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred to ensure they are aware of the testing event and impending results. [Important – Given the heightened state of alert for *Bsal* and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred, to be sure they are aware of the mortality event and that testing is underway, while results are pending. This notification allows them to consider additional surveillance or management actions to further protect wild populations.]

### **Questions**

Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state or province/territory? (If not, the PARC Disease Task Team may be able to assist; send a message to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org) requesting information on the appropriate contacts.) **For management agencies or industries:** Are there other partners that you need to engage, and if so, should it be at this stage or after results are received?

### **Recommended actions (in no priority order and as feasible):**

#### **1) Tissue collection for diagnostics.**

- a. Collect tissue and/or moribund (dying, unable to right themselves), abnormally behaving, or co-located live animals, as feasible and using humane euthanasia procedures, as applicable (Appendix I, Section A), for submission to the facility's



pathologist, where applicable, or, after confirming the closest lab able to handle the specific case, to a Participating Laboratory (see also the Diagnostic pages of [www.salamanderfungus.org](http://www.salamanderfungus.org)).

- b. Collect carcasses, fresh-dead animals, for diagnostic necropsy; submission to Participating Laboratory (Appendix I, Section A).
  - c. Consider collecting swabs from living animals without signs of disease contained in the same enclosures or nearby.
- 2) **Biosecurity protocols**, as established in Pessier and Mendelson (2017), implemented for:
- a. Disinfection of captive caging/housing facilities and materials prior to reuse for treated or new animals.
  - b. Treatment and disinfection of water prior to disposal.
  - c. Treatment of plant or soil substrate materials prior to disposal.

**Question**

Have you established/considered establishing an approved set of biosecurity protocols for disease-affected population/housing materials in captivity?

- 3) **Containment considerations.** For exposed captive animals that remain living, we suggest the following:
- a. Individual quarantine for all potentially exposed animals until causative agent is determined.
    - i. Consult with your local amphibian or veterinary expert and consider prophylactic treatments, and post-treatment testing and monitoring, as per guidance in Blooi et al. (2015a,b).
  - b. Halt transport/commerce of exposed, co-located, co-shipped, or all amphibians until health conditions and pathogen eradication can be verified.
  - c. Retrieve chain-of-contact/custody information (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
    - i. Inform all personnel at potential points of transmission and recommend they follow quarantine, testing, and treatment recommendations.

- d. Ensure biosecurity standards have been met (see action #3) prior to resumption of any transport or commerce of animals or caging materials, in accordance with existing federal, state or provincial/territorial, or local laws.
- 4) See “Definitive detection, **Captive**” scenario below for additional responses.

### Scenario 3: Detection of *Bsal* Presence by Polymerase Chain Reaction (PCR) (Wild or Captive)

**This scenario is defined as:** Detection of *B. salamandrivorans* DNA, as determined by a Participating Laboratory, based on PCR testing of swab or tissue samples of individual amphibians or samples from the environment (e.g., environmental DNA [eDNA] sampling). [Ideally, the Participating Laboratory will have also verified the result by a second Participating Laboratory.] This scenario indicates potential presence of *Bsal*, but it is NOT considered a “definitive detection” of *Bsal* until additional evidence of *Bsal* has also been determined. The guidance below is to facilitate early detection, rapid response efforts while confirmation of *Bsal* presence is pending.

[A detection of *Bsal* presence via PCR could occur a) in an instance where no clinical sign or histopathologic evidence, nor evidence of a current mortality event, exists that is indicative of an active *Bsal* outbreak, b) as an outcome of Scenarios 1 or 2 above, or c) independently via surveillance or research of wild or captive populations.]

#### Actions recommended (one or more, as feasible):

- 1) Initial diagnostic results communicated by Participating Laboratory to:
  - a. Reporting Individual(s), who in turn informs:
    - i. Detection site landowner/manager
    - ii. Wildlife agency or entity with management authority
- 2) Agency or entity with management authority forms and convenes the CRT. [Some entities customizing this template may consider developing an incident command system to help coordinate across other agencies or stakeholders.]
  - a. Consider also engaging the *Bsal* Task Force Technical Advisory Committee (TAC) leadership ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)), who are available to assist by advising on resources and responses, and will keep the information confidential. [Through the Task Force’s working groups, additional assistance can be provided on next steps following a PCR detection.]
  - b. Consider developing a communications plan that facilitates internal agency and CRT communications to external stakeholders and the public (including signage for affected sites and intended visitor behavior modifications). [These are potential, suggested components of a communications plan; customized actions may differ.]

#### Questions

Is there any cultural or archaeological significance of the site?  
Is it a popular visitor site that may require a visitor management plan or additional staffing to advise the public and help avoid disturbance or public contact with affected areas?

**3) Further investigation.** Additional diagnostic testing should be conducted as feasible (e.g., sequencing and phylogenetic analyses, isolation by fungal culture, necropsy, and histopathologic examination of associated dead animals or tissues where applicable) by a Participating Laboratory for a definitive diagnosis (White et al. 2016).

**4) Management actions, *wild populations***

- a. Biosecurity protocols, as established (Appendix I, Section A(3)), implemented for all field gear used at the *Bsal*-positive site.
- b. Increased surveillance at the *Bsal*-positive site.
  - i. If available, test any archived amphibian tissues from the site of detection for *Bsal*.
  - ii. Evaluate known amphibian species composition at the site, with special consideration for the presence of federally-listed, state-listed, and at-risk salamander species.
    1. If listed and/or at-risk species are present, evaluate the need and opportunity available for taking healthy individuals from the wild and placing them in captivity for establishment of a breeding (captive assurance) colony.
  - iii. Conduct additional sampling of amphibians and water at the site of detection.
  - iv. Evaluate movements of other animals in or out of the site
- c. Heightened awareness by managers at the *Bsal*-positive site.
  - i. Collect any morbid or dead amphibians at that site and submit them to Participating Laboratory for testing.
  - ii. Review any existing data from the vicinity of the site for evidence of population or mortality trends.
  - iii. Initiate population monitoring of affected amphibian species to determine if the population is stable or declining.
- d. Containment considerations. Consider options that might help prevent the spread of *Bsal*:
  - i. Restricted public access to the exposed area(s).
  - ii. Signage at or around the exposed area(s).
  - iii. Local personnel notification and access restrictions to the exposed area(s).

Direct actions should consider risk from multiple perspectives, and assessment should extend beyond the immediate area of concern. Consider working with decision scientists to take effective actions in the context of multiple management objectives and varying risk profiles.

**Questions**  
 [add as needed]  
 Is drying or treating the site an option? Is the harm of taking an extreme action greater than doing nothing?

**5) Management actions, *captive populations***

a. Containment:

- i. Ensure no shared water sources or water flowing out of the affected animals' caging/housing.
- ii. Individual quarantine. Isolate affected animals and any others that were housed with affected individuals.

- 1. Perform additional diagnostics on co-located individuals.
- 2. Eradicate *Bsal* sources.

a. For live, captive animals whose samples return a positive *Bsal* result, eradication may be attempted:

- i. For fail-safe eradication, we recommend humane culling or euthanasia. See Section 8.6 in Pessier and Mendelson (2017) or humane methods in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals ([AVMA 2020](#)) and either:

- 1. Preservation of infected individuals for further histological analysis (consult with your CRT and your Participating Laboratory to confirm necessity).
- 2. Disposal of infected individuals using strict biosecurity protocols.

- ii. If there are reasons to maintain the animals, eradication of *Bsal* may be possible and has been demonstrated in published literature (Bloom et al.

2015a,b). [There may be reasons to maintain and treat animals, e.g., with threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.]

1. Treat per guidance in Blooi et al. (2015a,b). [As new treatments and research are being investigated, we will update this template. **Please note:** The methods tested to date have only been confirmed in Fire Salamanders (*Salamandra salamandra*); keep in mind that species differences may come into play with respect to treatment validity and effectiveness, which is why multiple swabs for PCR testing over time are necessary to confirm eradication.]
  2. Swab treated animals post-treatment (see Appendix I, Section B) and submit repeat samples to a Participating Laboratory to confirm *Bsal* eradication.
  3. Repeat treatment regime(s) and post-treatment swabbing until confirmation of *Bsal* eradication.
- b. Disinfection, per Pessier and Mendelson (2017), of:
- i. All caging/housing materials and equipment prior to reuse.
  - ii. All water prior to disposal.
  - iii. All plants, soils, or other organic materials prior to disposal.
- c. Captive population monitoring. Evaluate the exposure of other co-located amphibians, including:
- i. Determine other places *Bsal* could be in the facility and disinfect those areas.
  - ii. Assess other potential sources of spread or origin of the pathogen, including through shared water sources, and quarantine or disinfect these sources.

- iii. Assess the entire population to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
  - d. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
    - i. At minimum, swab amphibians for PCR analysis throughout the chain of custody.
    - ii. Consider additional monitoring, as in 5(c) above.
- 6) Document *Bsal* treatment.** Prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying *Bsal* treatment and eradication for *each individual animal* that tested positive for *Bsal* and was treated and for which *Bsal* was shown to be eradicated. [Entities customizing this template should keep in mind that each state may or may not have specific laws regarding “official” health certifications or alternative options; it is important to consult your state fish and wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.]

**7) Additional management guidance via CRT**

- a. Messaging considerations.
  - i. CRT will advise on and assist in development of preliminary detection messaging for the Reporting Individual(s) or the agency/entity with management jurisdiction over the site of detection to disseminate information.
- b. Movement restrictions, voluntary or mandatory, implemented by landowner/manager, captive population owner, or agency with jurisdiction over the captive animals, to reduce further transmission (e.g., prohibitions on collecting salamanders from the wild site; temporary moratorium on movement or sale of salamanders from the captive facility until further information is known). [Entities customizing this template may consider including additional guidance for tracking animals that were documented to be infected and then treated, including reporting or other requirements upon relocation to new jurisdictions.]

**8) Subsequent communications:**

- a. If the *Bsal* Technical Advisory Committee has not been engaged in prior steps, consider contacting them regarding the findings and actions ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)).

- b. Internal communications as required by the Reporting Individual's agency/organization.
- c. Internal communications within the agency or entity with management jurisdiction of the detection site as management decisions are made, on a need-to-know basis.
- d. Local stakeholder and chain-of-contact/custody outreach.
- e. No further communications until detection status is definitive. [Limiting communications to a "need to know" group of people until confirmations of *Bsal* (or other pathogen) detection is received, may help to avoid unnecessary attention or public reaction.]



**Scenario 4: Definitive detection, Wild**

**This scenario is defined as:** Evidence of *both* 1) the **presence of *Bsal***, as determined by the Participating Laboratory through either PCR-testing or isolation of a *Bsal* fungal culture as identified with genetic sequencing *and* 2) **histopathologic lesions** that confirm *Bsal* infection as the cause of disease or mortality.

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**IMPORTANT**

Evidence of *Bsal* presence without confirmation with a second diagnostic test or demonstration of histopathologic lesions is not enough to determine definitive detection of *Bsal* chytridiomycosis (see Iwanowicz et al. 2017). Interpretation of laboratory results should follow the case definition for *Bsal* chytridiomycosis (White et al. 2016) accepted by the Diagnostics Working Group of the *Bsal* Task Force.

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**Actions recommended (one or more, as feasible):**

- 1) Results communicated by Participating Laboratory to:
  - a. Reporting Individual(s), who in turn informs:
    - i. Detection site landowner/manager
    - ii. Wildlife management agency with jurisdiction over species and/or land
- 2) Agency or entity with management authority forms and convenes the CRT.
  - a. Consider also engaging the *Bsal* Task Force Technical Advisory Committee leadership ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)), who are available to assist by advising on resources and responses and will keep the information confidential.
- 3) Subsequent communications (in order of priority):
  - a. Internal communication as required by the Reporting Individual's agency/organization.
  - b. Notification to the *Bsal* Task Force Technical Advisory Committee leadership ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)), if they have not yet been informed.
  - c. Formal stakeholder notifications (e.g., partner institutions or agencies).
  - d. Public announcement/press release as appropriate.
  - e. Local stakeholder outreach (e.g., public groups who use the affected sites and could be asked to disinfect gear and report observations of dead amphibians).

- f. Statement in scientific publication outlet.
  - g. Entry into *Bsal* reporting database.
- 4) Emergency meeting convened among parties identified in 2a and possibly 3a–b above to discuss:
- a. Risk/threat assessment. [Some areas to assess for potential risk include species movements, people’s activities, water movements, etc. and risk level to co-occurring species.]
  - b. Management actions and considerations:
    - i. Containment of mortality/detection site:
      - 1. Landowner/manager restrictions on public access to site, except for approved personnel.
      - 2. Strict use of approved biosecurity protocols (Appendix I, Section A(3)) for all personnel, their gear, vehicles, etc. when exiting site.
        - a. Establish dedicated equipment/gear, including nets, footwear, etc., for the site.
      - 3. Deployment of fencing or other containment measures to reduce or prevent spread by other wildlife.
      - 4. Demarcation of the affected area(s) to minimize or prevent trespass by personnel or public.
    - ii. Establishment of ex situ colony(-ies):
      - 1. Engage additional partners (Amphibian Ark, CAZA, AZA, American Association of Zoo Veterinarians, etc.) to assist.
      - 2. Initiate rescue/captive assurance populations:
        - a. Based on conservation status (e.g., federally or state/provincially-listed).
        - b. Based on proportion of local population affected and proportion of total population represented locally.
        - c. As an attempt to salvage/save affected but treatable individuals.
    - iii. Priority surveillance:
      - 1. Detection site:

- a. Sampling of other amphibian species at the detection site, particularly any within those families shown to be susceptible in Martel et al. 2014 and Stegen et al. 2017 (or more recent publications, if available).
    - b. Additional sampling of exposed amphibian species or substrates.
  2. Non-independent sites (e.g., potential transmission pathways of water bodies connected to the detection site by permanent or ephemeral water flow or watershed considerations as well as adjacent terrestrial areas).
  3. Adjacent waters or lands within natural movement distances of the affected species.
  4. Nearby sites that may serve as refugia for translocating uninfected salamanders.
- iv. Movement restrictions and prohibitions on collections of wild salamanders from the affected site.
- v. Other interventions as feasible, e.g., antifungal treatments for surviving animals, as described by Blooi et al. (2015b), or possibly habitat treatments or disinfection. [As new information becomes available on pending research and mitigation strategies, we will update this template. Preliminary data show some habitat treatments may be effective in eradicating the related pathogen, *Batrachochytrium dendrobatidis* (*Bd*; Bosch et al. 2015). In the early stages of *Bsal* detection and rapid responses, the following treatments may be the best options to attempt containment and local eradication at site-level habitat:
1. Culling/euthanasia
  2. Bleaching site
  3. Draining
  4. Site closures (including physical barriers)
  5. Signage or additional staffing to address desired visitor behavior modifications]

### **Questions**

Whom might you contact for each of the above possible actions? Is there an “expert team” you could develop and have on call for the different actions above? The *Bsal* Task Force can assist in identifying a few national contacts, and perhaps also some local contacts, as a start.

What local, state or provincial/territorial, or federal resources are there to accomplish the actions above (e.g., laboratories, chemical application, or water draining equipment)?

What local, state or provincial/territorial, and federal laws may apply for environmental compliance? Do agency or local law enforcement contacts need to be informed or engaged?

### Scenario 5: Definitive detection, **Captive**

**This scenario is defined as:** Evidence of *both* 1) the **presence of *Bsal***, as determined by the Participating Laboratory through either PCR-testing or isolation of a *Bsal* fungal culture as identified with genetic sequencing; *and* 2) **histopathologic lesions** that confirm *Bsal* infection as the cause of disease or mortality. [Evidence of *Bsal* presence without confirmation with a second diagnostic test or demonstration of histopathologic lesions is not enough to determine definitive detection of *Bsal* chytridiomycosis (see Iwanowicz et al. 2017). Interpretation of laboratory results should follow the case definition for *Bsal* chytridiomycosis (White et al. 2016) accepted by the Diagnostics Working Group of the *Bsal* Task Force.]

#### **Actions recommended (one or more, as feasible):**

- 1) Results communicated by Participating Laboratory to:
  - a. Reporting Individual(s), who in turn informs:
    - i. Captive animal owner/captive facility manager or veterinarian
    - ii. State or provincial/territorial agency(-ies) with jurisdiction over captive animal health and movement (e.g., wildlife management agency or state/provincial/territorial department of agriculture)
- 2) Agency or entity with management authority forms and convenes the CRT.
  - a. Consider also engaging the *Bsal* Task Force Technical Advisory Committee leadership ([response@salamanderfungus.org](mailto:response@salamanderfungus.org)), who are available to assist by advising on resources and responses, and will keep the information confidential.
- 3) Subsequent communications (in order of priority):
  - a. Internal reports within agency/organization (if applicable).
  - b. Notifications to pet store, or importer, or zoological institution where animals were acquired.
  - c. Notifications to chain-of-contact/custody stakeholders (i.e., individuals or entities throughout the history of possession of the affected amphibians and other associated individuals or entities).
  - d. Formal stakeholder notifications (per CRT guidance).
    - i. State veterinary health official.
    - ii. AZA Taxonomic Advisory Group or Species Survival Plan contacts.
  - e. Statement via scientific publication outlet.
  - f. Entry into *Bsal* reporting database.

- g. Public announcement/press release as appropriate (and in collaboration with captive animal/facility owner).
- 4) Emergency meeting convened among parties identified in 2 and possibly 3(a–c) above to discuss:
- a. Risk/threat assessment.
  - b. Management actions.
    - i. Containment.
      - 1. Ensure no running water out of the animals' housing area.
      - 2. Eradicate *Bsal* sources.
        - a. For live, captive animals whose samples return a positive *Bsal* result, eradication may be attempted:
          - i. For fail-safe eradication, we recommend humane culling or euthanasia and disposal of infected individuals using strict biosecurity protocols. See Section 8.6 in Pessier and Mendelson (2017) or AVMA Guidelines for the Euthanasia of Animals ([AVMA 2020](#)).
          - ii. If there are reasons to maintain the animals, eradication of *Bsal* may be possible and has been demonstrated in published literature (Bloom et al. 2015a,b). [There may be reasons to maintain and treat animals, e.g., threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.] In such instances, we suggest the following:
            - 1. Treat per guidance in Bloom et al. (2015a,b). As new treatments and research are being investigated, we will update this template. **Please note:** The methods tested to date only are confirmed in Fire Salamanders (*Salamandra salamandra*); keep in mind that species differences may come into play with respect to treatment validity and effectiveness, which is why multiple swabs

- over time are necessary to confirm eradication.
2. Swab treated animals post-treatment (see Appendix I, Section B), and submit samples to a Participating Laboratory to confirm *Bsal* eradication.
  3. Repeat treatment regime(s) and post-treatment swabbing until confirmation of *Bsal* eradication.
- ii. Quarantine. Isolate any potentially affected individual animals, including any that were housed nearby or co-located with affected individuals.
1. Perform additional diagnostics on quarantined, co-located individuals.
  2. Employ strict use of biosecurity protocols (see Pessier and Mendelson 2017) for all people/personnel handling the affected species, particularly prior to exiting the quarantine area.
- iii. Disinfection, per Pessier and Mendelson (2017):
1. All caging/housing materials and equipment prior to reuse.
  2. All water prior to disposal.
  3. All plants, soils, or other organic materials prior to disposal.
- iv. Captive population monitoring. Evaluate the exposure to other co-located amphibians, including:
1. Determine other places *Bsal* could be in the facility, and disinfect these areas.
  2. Assess other potential sources of spread or origin of the pathogen, including through shared water sources, and quarantine or disinfect these sources.
  3. Assessment across the captive population to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
  4. Evaluate other sources of infection, including new acquisitions.

- v. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
  - 1. At minimum, swab amphibians for PCR analysis in either direction throughout the chain of custody.
  - 2. Consider additional monitoring, as in 4(b)(iv) above.
- vi. Voluntary surveillance of affected populations.
  - 1. Additional sampling of affected species and captive environment (plants and other substrates).
  - 2. Sampling of all other amphibian species in the facility.
  - 3. Sampling of stock of original importer or zoological collection
    - a. Exposed animals
    - b. Other co-located animals
  - 4. Sampling throughout the chain-of-contact/custody of exposed individual animals.
- vii. Voluntary movement restrictions/prohibitions of movement or sale of affected species.
  - 1. Place a temporary moratorium on the sale or movement of all salamanders from the same zoological collection, captive breeder, pet supplier, or importer. [The entity customizing this document can determine whether to qualify this action as “mandatory” or “required” or use another descriptor. When there is a definitive detection of *Bsal*, we suggest the strongest possible measures to reduce risk of spread and facilitate containment.]
  - 2. Document *Bsal* treatment. If animals are treated prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying *Bsal* treatment and eradication for *each individual animal* that tested positive for *Bsal* and was treated and for which *Bsal* was shown to be eradicated. [Entities customizing this template should keep in mind that each state may or may not have specific laws regarding “official” health certifications or alternative options; it is important to consult your state fish and



wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.]

## **Appendix 4.1**

### **PROTOCOLS AND PROCEDURES FOR SAMPLING FROM MORTALITY EVENTS, AND FOR SAMPLING FROM LIVING ANIMALS, FOR DIAGNOSTIC TESTING**

Text adapted, with permission, from:

Pessier, A.P., and J.R. Mendelson III (Eds.). 2017. A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs, version 2.0. IUCN/Species Survival Commission (SSC) Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA. (Available from: <https://www.cpsg.org/disease-manual-amphibians-update-2017>)

**[NOTE: This appendix will be updated to reflect any new information as it becomes available.]**

**a. Tissue collection during mortality events.** Mortality events where multiple animals are found dying or dead are observed in amphibian survival assurance colonies as well as wild amphibian populations. Although well-known infectious diseases of amphibians (e.g., chytridiomycosis or *Ranavirus* infection) may be strongly suspected, it is important to keep an open mind and always consider other potential causes. Many different disease conditions can initially look very similar and require laboratory investigation to achieve a definitive diagnosis.

The initial goal of investigating mortality events is to collect and preserve representative samples that can be used for the different types of laboratory techniques that may be needed. It is always advisable to contact the lab where you intend to send samples and discuss with them their preference on how to prepare and ship the animals. [\[If possible, well in advance of a mortality event, consider contacting your nearest diagnostic laboratory to find out their preferences for preparing and shipping animals or samples in various scenarios of a mortality event.\]](#) Complex protocols can be designed for sample collection during mortality events—especially if veterinary guidance is available. However, a simple and basic approach is also sufficient for most situations.

- If wildlife health expert guidance is not available or if animals are small:
  - Perform the carcass-fixation necropsy method (see Chapter 9 in Pessier and Mendelson 2017) on one-half to two-thirds of the dead animals.
  - For the remaining animals, freeze the carcasses whole as soon as possible and label with the species name, individual identification number, and date.
    - For freezing of entire carcasses or individual tissue samples, ultracold temperatures ( $-70^{\circ}\text{C}$  or below) or liquid nitrogen are preferable. However, regular household freezer temperatures ( $-20^{\circ}\text{C}$ ) are sufficient for short-term storage.

- As a last resort, if a freezer or liquid nitrogen is unavailable, fixation of carcasses or tissue samples in 70% ethanol (instead of formalin) may still allow application of some molecular diagnostic techniques.
- If wildlife health expert guidance or an individual experienced with amphibian anatomy is available, perform the dissection necropsy method (see Pessier and Mendelson 2017) on the dead animals.
- In addition to saving samples from all major organs in fixative solution for histopathology, freeze additional samples of individual organs.
  - Suggested samples for freezing include skin, liver, kidney, lung, intestine, brain, and any tissue thought to be abnormal during dissection (e.g., enlarged or discolored organs or organ nodules). In addition, stomach contents, coelomic fat bodies, and skeletal muscle can also be saved, especially if exposure to a toxic substance is a possibility.
  - Organ samples are saved in sterile Whirl-Pak® style bags (Nasco, USA, [www.enasco.com](http://www.enasco.com)) or cryovials such as Nunc CryoTubes™ or Vanguard Cryos™ (Sumitomo Bakelite Co., Ltd., Japan, [www.sumibe.co.jp/english/](http://www.sumibe.co.jp/english/)).
  - Containers should be labeled with the species name, individual animal ID number, specimen type, date, and county and state where collected.
- If moribund (dying) animals are found, consideration should be given to humanely euthanizing some of these individuals for necropsy and sample collection (see Section 8.6 in Pessier and Mendelson 2017). This approach provides very fresh samples that are ideal for most laboratory methods used for disease investigation.

**1. Basic tissue sample collection protocol for amphibian mortality events** (wildlife health expert not available or in a field situation with limited equipment).

- For half of the dead animals, make an incision into the coelomic cavity and expose the internal organs.
  - For very small animals or if a knife is not available, just fix the carcasses intact.
  - Place the opened carcass into a fixative solution, such as 10% neutral buffered formalin (preferred) or 70% ethanol. The ideal ratio is one part animal carcass to nine parts fixative solution.
- For the other half of the dead animals, freeze the carcasses whole or keep them cool (such as in a portable ice-chest) until they can be transported to a location where freezing is possible.
  - It is always better to save both fixed (formalin or ethanol) and frozen samples. If this is not possible, preference should be given to saving tissues fixed in formalin or ethanol.
  - Saving only frozen samples should be a last resort (but is better than no samples at all).

- If freezing of samples is not possible, fixation in ethanol may allow for both histopathology as well as some molecular diagnostic tests (e.g., PCR)
- 2. Shipment of samples** (shipment of tissues that have been preserved in a fixative solution). Once carcasses or tissues have been in formalin or another fixative solution for a minimum of 48 hours, remove from fixative, wrap in paper towels or gauze moistened with fixative, pack into sealed plastic bags, and ship to a pathologist. This approach minimizes the potential for leakage during shipment and reduces package weight (and shipment costs).
- Materials should be shipped in a manner that follows International Air Transport Association (IATA) regulations for Dangerous/Hazardous Materials (see also <https://www.gpo.gov/fdsys/pkg/FR-2011-07-20/pdf/2011-17687.pdf>). Some general guidelines include:
    - Samples should be enclosed in a primary receptacle that is leak-proof.
    - The primary receptacle is then placed within a leak-proof secondary receptacle.
    - An absorbent material (e.g., paper towels) should be placed between the primary and secondary receptacles. The volume of material should be sufficient to absorb all of the fluid within the primary receptacle.
  - Major shipping companies have guidelines available to help with proper shipping of biological samples. More information available here: <http://images.fedex.com/downloads/shared/packagingtips/pointers>
- 3. Disinfection and biosecurity in the field.** Concerns about the possibility of moving amphibian pathogens to new locations as the result of field research conducted on wild amphibians have led to a number of protocols for reduction of this risk (e.g., <http://northeastparc.org/disinfection-protocol/>). There are variations and sometimes contradictions between the different protocols; however, the basic principles of biosecurity for biologists working on wild amphibian populations are similar. Peer-reviewed publications including the addition of risk calculators to assist the biologist in making good biosecurity decisions have recently become available (St-Hilaire et al. 2009; Phillott et al. 2010). A summary of recommended field practices includes:
- **Definition of the field site.** The first precaution against the possible spread of disease among amphibian populations is careful definition of the field site or sites. Researchers should use natural and man-made boundaries to help define the sites. Whenever possible, plans should be made ahead of time to work in only one site per outing or have different groups working at each individual site to avoid cross-contamination (and transmission of disease) between sites.
  - **On-site hygiene and biosecurity of equipment.** The use of disposable equipment discarded after use at a single site or on a single individual amphibian reduces the risk of spreading disease. All reusable equipment, including footwear, should be disinfected between sites or dedicated to a single site (e.g., a single pair of rubber boots is purchased for each field site

and used ONLY at that site). Consult the table in Section 5.10 of Pessier and Mendelson (2017) for details on the use of specific disinfectants, including recommended concentrations and contact times.

- Footwear and other reusable equipment should be made of materials that are easy to clean and disinfect (e.g., rubber boots are better than leather hiking boots).
- Thorough cleaning of equipment is essential for removal of dirt and organic material prior to disinfection in the field. As noted in other sections, organic material inactivates many disinfectants. Scrub brushes and other implements to remove dirt should be part of the field equipment. If disinfectant solutions become contaminated with organic material or dirt, they should be changed.
- The quaternary ammonium compounds (see Section 5.2 in Pessier and Mendelson 2017) have been recommended for field situations because they are concentrated and easy to transport into field situations (Johnson et al. 2003, Webb et al. 2007).
- If disinfection is undertaken in the field, consideration should be given to the toxicity of chemicals to the environment. The quaternary ammonium compounds and Virkon® (see Section 5.2 of Pessier and Mendelson 2017) are more environmentally friendly options compared to chlorine bleach (Johnson et al. 2003, Webb et al. 2007, von Rütte et al. 2009). If ranaviruses are a special concern, Virkon® may have some advantages over the quaternary ammonium compounds (Bryan et al. 2009). Powdered bleach is another easily portable suggestion.
- Vehicles are less likely to be a vector for the transmission of disease than are footwear and field equipment but still should be disinfected, especially if used to cross or enter a known contaminated site. The wheels and tires should be cleaned of all dirt and organic material and disinfected prior to leaving the site by using the same disinfectant that was used on footwear. Always remember to disinfect footwear before getting into a vehicle to prevent pathogens from transferring to the floor or pedals.
- **Handling and collection of samples from amphibians.** When handling amphibians in the field, even within the same site, precautions should be taken to minimize the risk of transmitting pathogens between individual animals.
  - Non-powdered disposable gloves are the best choice when handling amphibians. Powdered gloves should be rinsed free of powder. A new pair of gloves should be used for each animal. If gloves are unavailable, it is slightly preferable to use bare hands, and wash hands between handling different animals (Mendez et al. 2008).
  - The greatest risk for spreading disease when handling amphibians occurs when animals are placed together in the same container or

when containers are reused without being disinfected. Do not reuse collecting bags—utilize a new one for each animal.

- Always handle animals as little as possible. Procedures that are quick, even if potentially painful, may cause less stress than longer procedures.
- Animals should only be released at the site of capture, and any sick or dead amphibians found should be preserved in 10% buffered formalin solution and submitted for disease diagnosis (see Chapter 9, Necropsy, in Pessier and Mendelson 2017).
- Instruments used for sample collection should be disinfected between use on different animals. For surgical instruments (e.g., scissors) and weighing equipment, 70% ethanol is rapidly acting against the amphibian chytrid fungus (Johnson et al. 2003).
- Although mentioned in some amphibian handling protocols, the use of iodine-based compounds for sanitizing the animal's skin prior to procedures such as toe-clipping or microchip implantation is not recommended because of toxicity concerns. Potential substitutes include 0.75% chlorhexidine or 2 mg/L benzalkonium chloride (Wright 2001).

**b. Sample collection for *Bsal* PCR.** [As of this version, some of the sample collection options for *Bsal* have not yet been documented; this information is provided based on techniques used for *Batrachochytrium dendrobatidis* (*Bd*) and will be updated as new information becomes available.] Based on what is known for *Bd*, the PCR procedure can be performed using a variety of different sampling methods including skin swabs, water bath, and tissue samples (e.g., toe clip; Hyatt et al. 2007).

- Skin swabs. The skin swab procedure is simple, minimally invasive, and it samples multiple areas of the skin that may be infected with *Bsal* (increasing the likelihood that infected areas will be sampled). Skin swabs generally are the preferred sampling method for *Bsal* PCR.
- Water bath. Samples using the water bath procedure require immediate centrifugation or micropore filtration and are not practical in many settings.
- Tissue samples. Toe clipping is an invasive procedure with associated ethical concerns and has the disadvantage of sampling only a small portion of potentially infected skin.

**1. Materials needed for skin swabbing.** The materials listed below are general guidelines needed to perform the skin swab procedure for *Bsal* PCR using realtime or quantitative PCR (qPCR) methods. There may be differences depending on the preferences of the laboratory processing the samples and the environmental conditions under which the swabs are obtained.

- Powder-free latex or nitrile disposable gloves.
- Sterile applicators (“swabs”); see “Swab Selection” in Pessier and Mendelson (2017).
- 1.5 ml microcentrifuge tubes/cryovials.

Storage of dry swabs at controlled room temperature/refrigeration or freezing is preferred, but 70% ethanol is an alternative, especially if samples will be exposed to variable climate conditions, such as heat. Individual laboratories may have preferences about sample storage conditions; be sure to check in advance with the Participating Laboratory to which samples will be sent. For additional information, see the section on “Storage of Skin Swab Samples” below.

- 2. Swabbing procedure 101.** Several videos demonstrating swabbing and associated biosecurity and prevention of contamination have been developed:  
[https://amphibiaweb.org/chytrid/swab\\_protocol.html](https://amphibiaweb.org/chytrid/swab_protocol.html)
- 3. Avoiding cross-contamination of samples.** The PCR assays are very sensitive tests and can detect very small amounts of *Bsal* DNA. This sensitivity is good for detecting animals that have very low-level infections with *Bsal*, but it increases the risk that samples from a non-*Bsal* infected animal can have false-positive results if they become contaminated with even small amounts of *Bsal* DNA from an infected animal. Therefore, it is very important to take precautions to avoid sample cross-contamination. These precautions include:
  - Use a new pair of disposable latex or nitrile gloves for each animal handled for testing (Mendez et al. 2008).
  - Avoid contact of swabs (especially swab tips) with surfaces or substrates other than the skin of the animal to be tested.
  - If instruments are used to cut the tip of the swab into cryovials, use a freshly disinfected instrument for each sample.
    - To disinfect instruments for this purpose, dip in 70% ethanol followed by flaming under an alcohol lamp.
    - Avoid using bleach solutions for disinfection because doing so can degrade *Bsal* DNA in swab samples (resulting in false-negative tests; Cashins et al. 2008).
- 4. Avoiding PCR inhibitors in samples.** Foreign material, such as dirt or plant matter, can contain materials that inhibit the PCR reaction, which can result in a false-negative test result (i.e., animal is infected with *Bsal*, but it is not detected by the PCR test).
  - Prior to skin swabbing, efforts should be made to manually remove heavy skin contamination. Animals may be gently rinsed with clean water prior to sampling, but vigorous washing should be avoided because of the potential to also rinse off *Bsal*-infected skin cells or organisms.

- If rinsing is necessary, it is best if the water does not originate from the animal's enclosure or environment.
- Laboratories that perform PCR for *Bsal* should always use exogenous internal positive controls to detect PCR inhibitors (Hyatt et al. 2007).

**5. Storage of skin swab samples.** Storage of swabs after sample collection is an important consideration. Swabs can be stored air-dried or in 70% ethanol. Be sure to check in advance with the Participating Laboratory to which samples will be sent; individual laboratories may have preferences about sample storage conditions.

For air-dried swabs, the major concern is high temperature extremes. DNA on air-dried skin swabs has been experimentally proved to be remarkably stable. Hyatt et al. (2007) demonstrated that PCR sensitivity was unaffected by storage of skin swabs for up to 18 months at room temperature (23°C). However, exposure of swabs to high temperatures (>38°C) for as little as seven days can result in decreased recovery of pathogen DNA, thus increasing the possibility of false-negative results in animals with low-level *Bsal* infections (Van Sluys et al. 2008). Therefore, it is recommended that air-dried skin swab samples be stored at the lowest temperature possible (Skerratt et al. 2008). The following are general guidelines:

- Store samples at 25°C (refrigerator) or lower.
- Samples should be frozen (–20°C or below) if sample analysis is not performed within six months of sample collection.
- See alternatives to low temperature storage (i.e., where refrigeration may not be possible) in Pessier and Mendelson (2017).

**6. Shipment of swabs to the laboratory.**

- Ideally, ship swabs by overnight or 2-day courier service (e.g., Federal Express; Canada Post Xpresspost, UPS, Purolator, etc.).
- Consider using cold packs to guard against high temperature extremes.
- Samples that have been previously frozen should be sent on dry ice to prevent freeze–thaw cycles.



## Appendix 5. North American *Bsal* Implementation Plan

**Update: 13 October 2020**

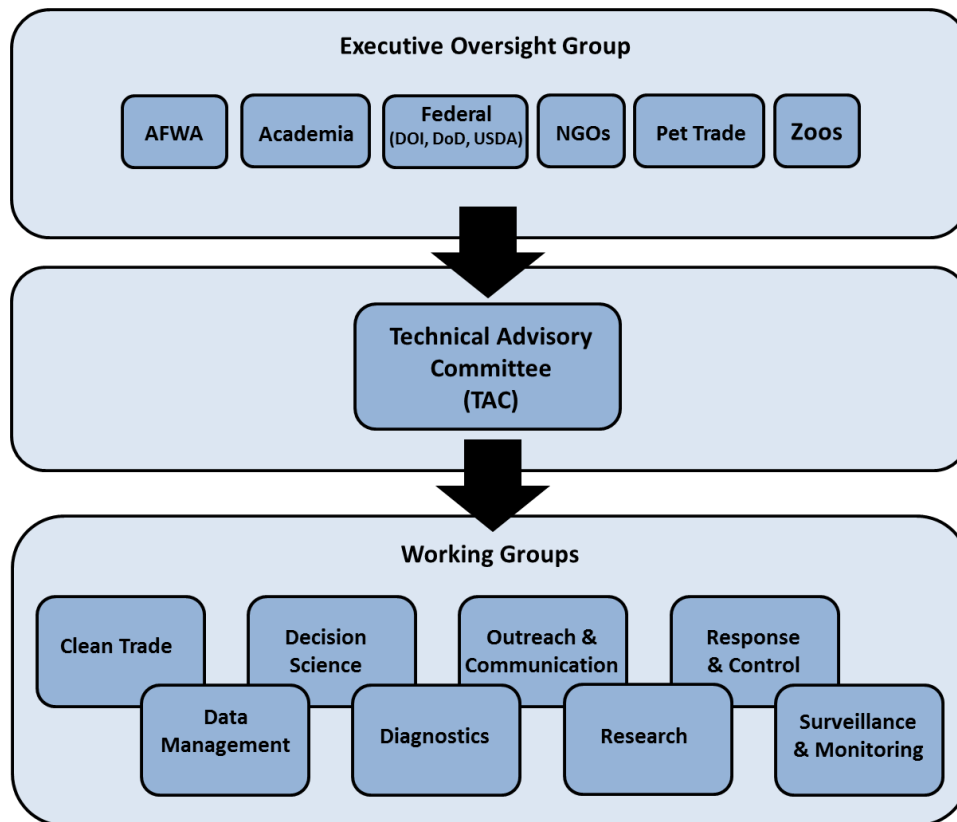
### Summary

The document “A North American Strategic Plan to Prevent and Control Invasions of the Lethal Salamander Pathogen *Batrachochytrium salamandrivorans*” (also referred to as the “*Bsal* Strategic Plan”, available at [salamanderfungus.org](http://salamanderfungus.org)) is the result of ongoing collaborations of members of the North American *Bsal* Task Force and partners. The overarching objective of the *Bsal* Strategic Plan is to provide interdisciplinary scientific and managerial guidance to forestall emergence of *Bsal* in North America. This is a complex objective that has led to the development of a Technical Advisory Committee (TAC) and multiple *Bsal* working groups. The TAC serves an oversight function for working groups and provides communication with key partners but also takes on overarching activities, such as collaborative projects among Task Force members and partners. Working groups are convened to advance knowledge relative to eight disciplinary themes and their integration: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade.

Actions of the TAC and working groups are developed in a hierarchical fashion outlined here in the North American *Bsal* Implementation Plan. First, the Framework of Actions summarizes the initial breadth of the Task Force’s aims as nine general intention statements. Second, goals of the TAC and working groups are articulated as statements that frame thematic activities specific to each group. Each of the nine statements in the Framework of Actions tiers to one or more goals shared among groups. Third, priorities of the TAC and working groups are specific tasks or activities undertaken under the theme of a goal. Priorities are the iterative steps that have been identified to advance achievement of the goal and may represent separate studies undertaken by working group members or subgroups or represent stages in the development of programmatic operations to advance *Bsal* response activities. As the *Bsal* Task Force has been in operation since 2015, some initial goals’ priority tasks have been completed. These completed tasks are briefly summarized below as well as the list of priorities in development at this time. The North American *Bsal* Implementation Plan is a living document intended to track current goals, priorities, and their separate tasks across working groups as well as for the TAC and *Bsal* Task Force as an overarching leadership cadre. This Implementation Plan is written as Appendix 5 of the *Bsal* Strategic Plan, with updates to be posted on partner web pages (e.g., [salamanderfungus.org](http://salamanderfungus.org); [amphibiandisease.org](http://amphibiandisease.org); [Partners in Amphibian and Reptile Conservation, national Disease Task Team web page](http://Partners in Amphibian and Reptile Conservation, national Disease Task Team web page)). Periodic updates are expected at annual or longer time scales and are noted by the date on the first page.

## Overview

The North American *Bsal* Task Force was initiated in June 2015 to address the complex facets of potential *Batrachochytrium salamandrivorans* (*Bsal*) emergence in North America and the projected dire outcomes that could ensue for native amphibian fauna because of this pathogen (Martel et al. 2014, Gray et al. 2015, Yap et al. 2015, Richgels et al. 2016). The document “A North American Strategic Plan to Prevent and Control Invasions of the Lethal Salamander Pathogen *Batrachochytrium salamandrivorans*” (also referred to as the *Bsal* Strategic Plan; [salamanderfungus.org](http://salamanderfungus.org)) summarizes interdisciplinary scientific and managerial guidance for a successful response to the detection of *Bsal* in North America. The *Bsal* Strategic Plan provides additional detail on the amphibian chytrid fungus *Bsal* (the pathogen that can cause the disease *Bsal* chytridiomycosis in some amphibians); emergence of the disease *Bsal* chytridiomycosis in Europe; current knowledge of the Asian origin of *Bsal*; and the origin, structure (Fig. A5.1), and aims of the *Bsal* Task Force.



**Figure A5.1.** Structure of the North American *Bsal* Task Force.

The Implementation Plan of the *Bsal* Strategic Plan is organized in a three-step hierarchical fashion. First, the **Framework of Actions** (Box A5.1) provides intention statements to capture the overarching breadth of the initial objectives of the Task Force. Second, **goals** describe the broad thematic aims undertaken by 1) the *Bsal* Technical Advisory Committee (TAC), the leadership body of the Task Force and its key partners, and 2) the working groups, assembled to

address separate disciplinary or operational topics in depth (Fig. A5.1). Goals of the TAC and working groups may tier to statements in the Framework of Actions. Currently, there are eight working groups: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade. Each working group has an objectives statement to define its scope, under which its goals define thematic activities. Third, to achieve the broad goals of the different groups, more specific **priorities** are developed. Priorities are independent task statements or activities that aid in a stepwise advancement of goals. Priorities may align with specific studies or operational procedures to advance the efficacy of the *Bsal* Task Force.

The North American *Bsal* Implementation Plan describes the objectives, goals, and priorities of TAC activities with its partners and each working group. Since 2015, several tasks have been addressed, and new goals, priorities and tasks have been developed over time. For each working group, a summary of completed tasks and current goals and priorities is provided.

The North American *Bsal* Task Force is a non-affiliated partner-based organization. To implement the goals, priorities, and tasks outlined in the *Bsal* Strategic Plan and Implementation Plan, in-kind resources of the myriad partners in North America (e.g., employers of Task Force members) are used. These resources include personnel time to advance various activities and, in some cases, operating and facility costs of projects undertaken. The *Bsal* Task Force is grateful for this support as well as for the volunteer hours donated to the Task Force's efforts. However, implementation of some work described below is heavily reliant upon obtaining external grants and agreements. As the work depending on funding from grants and agreements is an integral part of selected goal and priority implementation, the financial planning that accompanies those aims is briefly included in this document. In addition, as implementation decisions are weighed with consideration of limited available resources, the relative ranking of the importance of goals and priorities may be highly relevant. General ranks of Urgent, High, and Medium are provided for this purpose.

The North American *Bsal* Implementation Plan is intended to adapt over time as tasks are completed, priorities are met, new information is forthcoming, and new goals and priorities are identified by the TAC and the working groups. The Implementation Plan will be updated on associated web portals (e.g., [salamanderfungus.org](http://salamanderfungus.org); [amphibiandisease.org](http://amphibiandisease.org); [Partners in Amphibian and Reptile Conservation national Disease Task Team](http://Partners in Amphibian and Reptile Conservation national Disease Task Team)) and dated accordingly. Periodic updates are expected at annual or longer time scales.

### **Box A5.1. Framework of Actions**

Upon organization of the *Bsal* Task Force, nine guiding principles for strategic planning along several themes were developed to forestall *Bsal* emergence in North America. This Framework of Actions led to the development of eight Working Groups and associated collaborations among researchers, managers, industry, and other partners across North America.

1. Prevent invasion of *Bsal* into North America by encouraging stakeholders to work toward a clean trade program for amphibians that certifies individuals in trade are free of *Bsal* infection.
2. Develop and encourage use of the Rapid Response Plan (Appendix 4 in the Strategic Plan), which can be customized to meet local needs, to contain a *Bsal* outbreak.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of *Bsal* in animal or environmental samples in a timely manner.
4. Test for the occurrence of *Bsal* in Canada, Mexico, and the United States in the field and in captivity, reduce the risk of spillover from captive to wild amphibians, and reduce the likelihood of humans playing a role in the inadvertent translocation of *Bsal* within North America.
5. Advance the understanding of the risk of *Bsal* introduction to North America and assess the invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing *Bsal* surveillance data.
6. Develop effective, scientifically justified prevention and mitigation strategies that prevent *Bsal*-associated infections and mortality.
7. As evidence-based *Bsal* response and management actions are developed, identify expedited pathways for permitting actions and facilitate regulatory processes to implement mitigation measures.
8. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners that can communicate updates on *Bsal* developments and alert the public and scientific community if *Bsal* is detected in Canada, Mexico, or the United States.



Eastern Red-backed Salamander (*Plethodon cinereus*). © Alberto López.

## A. *Bsal* Technical Advisory Committee (TAC)

**Objective:** To serve as an integrated leadership body of the North American *Bsal* Task Force in collaboration with key partners, framing the scope and intent of the activities undertaken by the Task Force as a whole and as an advisory panel for working groups and ad-hoc subgroups.

**Leads:** María Forzán (Long Island University, NY, USA, current) and Molly Bletz (University of Massachusetts, MA, USA, in-coming)

**Past Leads:** Dede Olson (US Forest Service, OR, USA); Jennifer Ballard (formerly of US Fish and Wildlife Service, AR, USA); Mike Adams (US Geological Survey, OR, USA); Reid Harris (formerly of James Madison University and Amphibian Survival Alliance, VA, USA); Jake Kerby (University of South Dakota, SD, USA); and Matt Gray (University of Tennessee, TN, USA).

**Canada Liason:** Cynthia Pekarik (Environment and Climate Change Canada, QC, Canada)

**Past Canada Liasons:** Jennifer Provencher (Science and Technology Branch, ON, Canada); Sam Iverson (Canadian Wildlife Service, QC, Canada)

**Mexico Liasons:** Gabriella Parra-Olea and Eria Rebollar (Universidad Nacional Autonoma de México, Mexico)

The structure of the North American *Bsal* Task Force centers upon the Technical Advisory Committee (TAC) and the eight working groups (Fig. A5.1). The TAC is populated by the working group leads; representatives from Canada, Mexico, and the United States; and representatives from partner groups, including different government agencies, the IUCN Amphibian Survival Alliance (ASA), the Pet Industry Joint Advisory Council (PIJAC), and Partners in Amphibian and Reptile Conservation (PARC). The roles of TAC members vary considerably, with strategic surveillance, research, and proactive planning being tri-lateral priorities across the three countries and advances in biosecurity procedures being contributed by PARC's national and regional Disease Task Teams.

The TAC meets monthly by conference call, with a focus on new tasks of the TAC, progress reports on ongoing tasks, and round-robin reporting by working group leads and partners. New items have included actions or projects to be assigned or delegated to a subset of members, opportunities for products and grant proposals, subgroup activities with partners, and discussion of communication, outreach, and networking needs. A lead for the TAC is rotated each year,

adding broader perspectives to Task Force activities and ensuring shared leadership of developing products. The incoming and past leads help to transition work and knowledge. Decisions of the TAC are made by consensus. In particular, the TAC identifies novel implementation actions or priorities from new *Bsal* information forthcoming from the international *Bsal* research and management community, working groups, and partners.

**Goal A.1.** Leadership of the *Bsal* Task Force and ensuring progress toward the Framework Actions.

Rank: Urgent

Rationale: Coordinated oversight of the *Bsal* Task Force by working group leads and partners will aid inter-group communication and identification of strategic gaps in development of a program to forestall *Bsal* transmission to, or potential translocation within, North America.

Management Relevance: The Framework of Actions (Box A5.1) of the *Bsal* Task Force were identified as the initial scope of interdisciplinary work needed to understand and forestall potential *Bsal* emergence in North America and protect our natural heritage biodiversity into the future. To address these actions and additional concerns that have been raised in a coordinated fashion, the TAC and its partners and ad-hoc subgroups have taken on a variety of specific tasks that have helped the *Bsal* Task Force to be better defined and developed (nine current priorities listed below). The Urgent rank of this goal has expedited development of numerous strategic actions, 2015 to present, raising awareness among natural resource managers and expediting response processes among both management and science communities.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel; grants for publication costs, symposia, and workshops.

*Priority A.1.1:* Expand North American collaborations for *Bsal* science and management with broader academic, governmental, non-governmental, and conservation interest groups.

*Priority A.1.2:* Foster inclusivity and diversity in the North American *Bsal* Task Force structure, participants, and leadership, including enhancing representation across Canada, Mexico, the United States, and the First Nations and Indigenous peoples of North America; geographic or cultural diversity within nations; disciplinary expertise, including epidemiology, wildlife biology, wildlife health and veterinary sciences, ecology, trade, and zoos and aquariums; representation across employment sectors, including academic and research institutions, government agencies, non-governmental or non-profit organizations, and business and industry organizations; and additional human dimensions, such as gender, age, and ethnicity.

*Priority A.1.3:* Organize international *Bsal* symposia with European partners to focus on communication of science and management directions in North America and Europe, two continents with heightened *Bsal* emergence concerns.

*Priority A.1.4:* Organize focused workshops, in conjunction with international symposia or other relevant meetings, to leverage assembly of interested experts for advancing

knowledge across the spectrum of the framework of actions in two continents with heightened *Bsal* emergence concerns, North America and Europe.

*Priority A.1.5:* Formally publish the North American *Bsal* Strategic Plan with online access for facilitate communication and referencing capability.

*Priority A.1.6:* Distinguish the North American *Bsal* Implementation Plan from the *Bsal* Strategic Plan and *Bsal* annual reports, which iterate annual achievements and new activities.

*Priority A.1.7:* Update the *Bsal* Task Force website ([salamanderfungus.org](http://salamanderfungus.org)) to provide easier access to information and expand web pages per working group.

*Priority A.1.8:* Leverage expansion of *Bsal* communication and outreach efforts with other scheduled events and opportunities, including Amphibian Week, which is planned by PARC and held annually during the first full week of May.

*Priority A.1.9:* Provide support to all working groups as needed but especially for the newest working group, the Clean Trade Working Group, as it navigates assembly and task identification across the United States and Canada.

**Background:** Examples of past TAC implementation priorities under Goal A.1 that have already been completed and have advanced the Task Force development and mission include 1) an establishment report of the *Bsal* Task Force (Grant et al. 2015); 2) an overarching decision science framework for addressing *Bsal* emergence in North America, a product led by the Decision Science Working Group with broad collaboration by the TAC and partners (Grant et al. 2017; described further below); 3) completion of the *Bsal* Rapid Response Plan Template (Appendix 4, *Bsal* Strategic Plan), a product led by the Response & Control Working Group under advisement of the TAC and consultation with partners (Appendix 4 of the *Bsal* Strategic Plan; described further below); 4) a scenario-building exercise of responses if *Bsal* were to be detected in North America, led by US Geological Survey scientists with a subgroup of TAC members and partners (Hopkins et al. 2018); 5) a position paper describing the benefits of early release of information regarding *Bsal* occurrence in novel areas without adverse implications for later scientific publication opportunities of principal investigators involved in the discovery (Adams et al. 2018); 6) the North American *Bsal* Strategic Plan, the overarching document within which this Implementation Plan is Appendix 5 and which describes *Bsal* and its discovery, the Task Force initiation and structure, and the scope and interests of working groups; 7) *Bsal* Task Force annual reports of achievements, developed with the Outreach & Communication Working Group (2016 to present, available at [salamanderfungus.org](http://salamanderfungus.org)); and 8) development of early drafts of this *Bsal* Implementation Plan.

TAC members, in association with key partners, have developed products that contribute to TAC and working group goals. In particular, PARC's national Disease Task Team includes *Bsal* TAC members and has taken on an important additional communication and outreach function for the North American *Bsal* Task Force. For example, they have developed: 1) a call to action for *Bsal* in North America (Gray et al. 2015); 2) a one-page briefing paper on *Bsal* ([parcplace.org/wp-content/uploads/2017/08/BsalBrief.pdf](http://parcplace.org/wp-content/uploads/2017/08/BsalBrief.pdf)); 3) guidance for herpetological pathogen surveillance, with *Bsal* applications specified (Gray et al. 2017); 4) a Herpetofaunal Disease Alert System (Gray et al. 2018; [parcplace.org/resources/parc-disease-task-team/](http://parcplace.org/resources/parc-disease-task-team/)) to help with a rapid response to disease-related die-offs in North America, including potential *Bsal*-related die-offs; and 5) biosecurity guidance to forestall transmission of pathogens, including

*Bsal* (Julian et al. 2020). In addition, in 2019, *Bsal* TAC members and partners organized the First North American *Bsal* Symposium, which was held at the joint annual meetings of The Wildlife Society and the American Fisheries Society in Reno, Nevada (Pereira et al. 2020; videos of invited talks: <https://itunes.apple.com/itunes-u/ut-forestry-wildlife-fisheries/id494866284>). More recently, the TAC held a virtual conference, the North American *Batrachochytrium salamandrivorans* Task Force Inaugural Annual Meeting, where overviews of the goals and activities of the Task Force were presented to members, wildlife managers, and members of the public (<https://www.salamanderfungus.org>; accessed 10 May 2021).

The TAC also has helped in an advisory capacity with decisions regarding transitioning priorities of *Bsal* working groups as initial tasks were completed. For example, after the Rapid Response Plan Template (Appendix 4, *Bsal* Strategic Plan) was completed, the former Response Working Group moved to a new focus of developing mitigations in response to *Bsal* detection and expediting policies for permitting actions that may be necessary if *Bsal* is detected in North America, even if those actions are disruptive to wild populations and their habitats. Such actions include, but are not limited to, ground-disturbing activities that could occur with fencing of areas or water containment and chemical applications at field sites with *Bsal* detection. The need for this pro-active step was identified in part as the result of a scenario-building exercise led by TAC members from the US Geological Survey (Hopkins et al. 2018) when it was recognized that ground-disturbing activities to forestall *Bsal* transmission at known sites of first-emergence in North America could be federally funded and may require NEPA (National Environmental Protection Act) policy to be followed, and use of chemical applications at field sites may require Environmental Protection Agency Section-16 approval. Hence, a new focus of the former Response Working Group was influenced by TAC members involved in the scenario exercise, which included an interaction of multiple partners and working groups, such as members of the Surveillance & Monitoring and Response Working Groups. The name of the Response Working Groups was changed to Response & Control Working Group to reflect the new goals and priorities it was assuming. Additionally, the TAC concurred with the decision by the Surveillance & Monitoring Working Group to move to a new phase of United States national *Bsal* field site surveillance and monitoring with partners at colleges and universities once their initial goal, led by US Geological Survey partners, to analyze ~10,000 animals for *Bsal* across the United States had been completed. Lastly, a new Clean Trade Working Group was recently initiated after TAC members were invited to speak at an annual meeting of the herpetofaunal trade industry sponsored by PIJAC, which focused new attention on Framework Action 1 (Box A5.1). This working group is expected to expand efforts to address potential human-mediated transmission of *Bsal* into North America via trade markets.

**Goal A.2:** Develop and strengthen lines of communication between the *Bsal* TAC and national leaders to address risk and response to *Bsal* emergence in North America.

Rank: Medium

Rationale: Development of a network of relevant partners to address concerns for *Bsal* in North America includes establishment of communication networks to higher-level national leaders with jurisdiction over the natural heritage of species and biodiversity in Canada, Mexico, the United States, and the First Nations and Indigenous peoples of North America.



Management Relevance: An Executive Oversight Group (Fig. A5.1) is envisioned to be created at a higher level than the TAC as a mechanism to more formally communicate with diverse natural resource managers or wildlife health leaders working at higher organizational levels of the government, especially as new *Bsal* information with high relevancy for nations becomes evident. These higher-level managers or leaders potentially include national-scale personnel, such as staff in the US Departments of Interior and Agriculture; the Association of Fish and Wildlife Agencies (AFWA), which includes federal, provincial and state leaders; the Canadian Wildlife Service (CWS), the Mexican Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and PIJAC. The Medium ranking of this goal reflects the complexity of authorities that need to align to facilitate agreement to convene an Executive Oversight Group.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel.

*Priority A.2.1:* Work toward development of an Executive Oversight Group for communication to higher levels of natural resource management in North America.

*Priority A.2.2:* Maintain regular communication with national liaisons about *Bsal* Task Force or information developments.

*Priority A.2.3:* In particular, communicate with herpetofaunal, wildlife, epidemiological, or natural resource specialists in Mexico and nations of the Caribbean to inform them of *Bsal* Task Force or information developments that may be relevant to management of their natural heritage of amphibian species.

Background: The initiation of a *Bsal* Task Force Executive Oversight Group (EOG) was proposed to national leaders at the North American Wildlife and Natural Resources Conference in March 2016. Discussion there identified the need for such an oversight body to extend beyond *Bsal* and include other non-agricultural wildlife diseases with existing analogous task forces, such as white-nose syndrome in bats, as well as wildlife diseases without formalized task forces, such as sea star wasting disease. Although an EOG has not yet been formally convened to address multiple wildlife diseases, an EOG for non-agricultural wildlife diseases has been the topic of continued discussion and communications across groups and with leaders across jurisdictions and authorities since 2016. This topic also relates to a recognized gap in laws for wildlife health in the Canada, Mexico, and the United States. Although the US Animal Health Protection Act (7 USC § 109) covers agricultural wildlife health, there is no companion legislation for non-agricultural wildlife. Similarly, in Canada, the Health of Animals Act is targeted toward agricultural animal health, so the Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (WAPPRIITA) is used to control the spread of *Bsal* via controlling salamander imports instead. These are examples of issues that the EOG could address.

This aim of the *Bsal* Task Force TAC to engage an effective oversight group among relevant government agencies is an ongoing task. Currently, key national liaisons in different government sectors have been identified for communication, if relevant topics arise. The resulting structure of the *Bsal* Task Force (Fig. A5.1), envisioned to be a hierarchical structure with overarching counsel from the EOG, is informally occurring as wildlife health guidance continues to develop with key partners interfacing with the actions of the TAC and working groups. Priorities under this goal reflect ongoing activities in this arena.



Eastern Newt (*Notophthalmus viridescens*). © Alberto López.

## B. Working Groups

Eight *Bsal* working groups have overarching goals per disciplinary area or thematic topic, which address one or more statements of the overarching Framework of Actions (Box A5.1). Within each of these goals are more detailed priorities of focused activities. The following sections detail the implementation plans of the objectives, goals, and priorities for the Response & Control, Diagnostics, Research, Decision Science, Surveillance & Management, Data Management, Outreach & Communication, and Clean Trade Working Groups.

Working group membership is open and inclusive, but the groups were initially founded with persons involved with disease research, natural resource management in state or provincial/territorial agencies and other government agencies, environmental or conservation groups, non-governmental organizations (NGOs), and the pet industry. Each working group has one to three leads, who help to coordinate personnel, manage the workload, and participate in the *Bsal* Technical Advisory Committee (TAC).



Three-lined Salamander (*Eurycea guttolineata*). © Alberto López.

## 1. Response & Control Working Group

**Objective:** To provide guidance for the rapid response (including, but not limited to, eradication, containment, or other management and control responses) should *Bsal* be detected in North America.

**Working Group Lead:** Laura Sprague (US Fish and Wildlife Service, ID, USA)

**Past Working Group Lead:** Priya Nanjappa (formerly of Association of Fish and Wildlife Agencies, Partners in Amphibian and Reptile Conservation, Washington, DC)

**Background:** Natural resource managers are often faced with making rapid decisions. The process can be quite overwhelming and confusing when dealing with multiple factors, like an emerging pathogen, species with conservation status of concern, and differing state or provincial/territorial and federal jurisdictions, regulations, policy, and permitting. The *Bsal* Response & Control Working Group aims to bridge the gap between identifying and implementing scientifically-sound mitigation actions in response to a confirmed *Bsal* detection in North America by proactively designing guidelines, identifying permitting steps, and facilitating the process of navigating the requirements for state or provincial/territorial and federal policy. Simply put, the purpose of the group is to facilitate efficient and rapid response to *Bsal* invasion.

In 2015, an initial Response Working Group focused on development of a *Bsal* Rapid Response Plan Template, addressing implementation Framework Action 2 (Box A5.1). After comprehensive review by federal, state, provincial, and other potential users of the Response Plan, this task has been completed, and the final product is included as Appendix 4 of the *Bsal* Strategic Plan (see [salamanderfungus.org](http://salamanderfungus.org)). The contents of the Rapid Response Plan are meant to be customized by any agency or institution with management jurisdiction over wild or captive salamanders so that the plan may serve as a template for actions required in case of suspected or confirmed *Bsal* detection.

The Rapid Response Plan Template is provided as an outline and guidance for local rapid response actions that could be triggered upon initial or subsequent detections of *Bsal*, in either

wild or captive populations. The scenarios involve different levels of diagnostic information for sick or dead animals. In other words, all recommended actions occur after the laboratory has made its determinations based on the *Bsal* case definition (White et al. 2016). Proactive actions to forestall *Bsal* emergence are not considered in this Rapid Response Plan Template but are being considered by the Decision Science Working Group, which is developing guidelines for implementing management actions before an introduction or outbreak is suspected or confirmed. The Rapid Response Plan Template provides considerations for in situ containment (i.e., in the existing location of the population) as well as establishment of ex situ populations (i.e., outside of the natural location, such as in a captive assurance colony). Rapid containment and response measures may prevent broad impacts of the infection. The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact the ARMI Decision Science Lead, Dr. Evan Grant ([ehgrant@usgs.gov](mailto:ehgrant@usgs.gov)), for assistance. The Rapid Response Plan is considered a living document that will be updated as more information becomes available. Updates will be posted on relevant websites (e.g., [salamanderfungus.org](http://salamanderfungus.org)).

After completion of the Rapid Response Plan Template (Appendix 4) and development of scenario-building exercises (Hopkins et al. 2018, Canessa et al. 2020), it became apparent that several aspects of responses to *Bsal* detection in North America warranted further development. These are tasks included in current priorities listed below.

**Bsal Mitigation:** In 2018, the newly reconfigured Response & Control Working Group was formed to continue and expand the work described above. Specifically, the Response & Control Working Group aims to facilitate an efficient and effective response, by development of *Bsal* control mitigations, if *Bsal* were to be detected at a field or captive site in North America. This effort is part of Framework Actions 6 and 7 (Box A5.1), which also intersect the aims of other working groups, such as the Research and Decision Science Working Groups.

Mitigation strategies can target the host or environment. We can use what we have learned from *Bd* as a foundation for developing and understanding potential disease mitigation and treatment strategies and also take advantage of novel directions as new, innovative ideas are discovered through research. Host-directed strategies are mitigation tools aiming to foster disease resistance or tolerance, such as skin probiotics (Bletz et al. 2013; Harris et al. 2009a,b), vaccinations (McMahon et al. 2014; La Patra et al. 2015), and antifungal medications (Hudson et al. 2016; Hardy et al. 2015; Bosch et al. 2015). Environment-directed strategies include micropredator manipulations (Schmeller et al. 2014; Buck et al. 2011), salt augmentations (Stockwell et al. 2015a,b), environmental probiotics (Muletz et al. 2012), habitat alterations (e.g., fencing to reduce animal movements and human encroachment, water diversions to preempt *Bsal* transmission with water flow), and removal of infected hosts. These strategies have potential for mitigating *Bsal*'s impact on North American salamander biodiversity.

To implement *Bsal* management actions on the ground, government agencies may be required to follow national and/or state/provincial/territorial policies related to the potential environmental impacts resulting from such actions. For example, during scenario exercises of potential *Bsal* outbreaks in the United States (e.g., Hopkins et al. 2018), rapid responses were identified to be potentially slowed by lack of permits for United States federal or state authorities to assist in field responses including ground-disturbing activities, disturbance to species with conservation status of concern, and use of chemical applications to address the *Bsal* fungus viability. The Response & Control Working Group activities currently focus on understanding

the permits and approvals that may be needed for various *Bsal* actions in field or captive settings and expediting rapid permit processes. An effort is underway to gain a better understanding of the complex network of permits and procedures in the United States and the processes that may expedite those approvals. Similar initiatives in Canada and Mexico are anticipated. Existing policies and procedures for the United States federal and state lands are described below.

**United States Federal Lands:** United States federal agencies are required to follow the policies of the National Environmental Policy Act (NEPA) of 1970. The NEPA process or “Environmental Impact Assessment process” applies when a federal agency has discretion to choose among one or more alternative means of accomplishing a particular goal. It requires agencies to determine if their proposed actions have significant environmental effects to land and water, protected wildlife and plants, historic properties, cultural resources, and other interests as well as to consider the environmental and related social and economic effects of their proposed actions. NEPA’s procedural requirements apply to a federal agency’s decisions for a variety of actions, including, but not limited to, permanent or temporary construction projects, limiting public access to public lands, using chemical or biological treatments, and permitting of private actions.

In the United States, private and state entities will often become involved in the NEPA process when applying for permits if they will be using public land access or public waters in their actions. The NEPA process is generally a long, drawn-out process that can be difficult to navigate if you are not familiar with it and can take years to accomplish, but the process must be completed before federal management decisions are made.

The Council on Environmental Quality (CEQ) oversees the NEPA process with the help of the Environmental Protection Agency (EPA), which issues permits for chemical and biologic use based on the Clean Water Act and Clean Air Act.

Once a proposed action has been developed, an agency can pursue one of two paths:

1. Environmental Assessment (EA), which determines the significance of the action’s effects and finds alternative measures.
2. Environmental Impact Statement (EIS), which must be prepared if an action significantly affects the quality of the human environment.

If an action may occur more than once, or it occurs routinely, and will not have a significant impact on the human environment (either positive or negative), the agency may seek a categorical exclusion (CE) from CEQ that precludes the need to prepare an EA or EIS for future actions, but the process for obtaining approval from CEQ for a CE is lengthy and complex. The need must be carefully justified, and CEs are rarely granted. However, on rare occasions, CEQ may exempt an action from NEPA under the following circumstances:

1. If the agency needs to take an action in response to an emergency, and the action would typically require preparation of an EIS, but there is insufficient time to follow the regular NEPA process, then the agency can work with CEQ to develop alternative arrangements for compliance with NEPA (40 CFR § 1506.11) and proceed immediately to mitigate harm to life, property, or important resources.
2. The NEPA analyses and document may involve classified information. If the entire action is classified, the agency will still comply with the analytical requirements of NEPA, but the information will not be released for public review. If only a portion of the

information is classified, the agency will organize the classified material so that the unclassified portions can be made available for review (40 CFR § 1507.3(c))

**United States State Lands:** There are currently 16 states with Environmental Quality Acts that require state and local agencies to perform EISs or at least Environmental Reviews (ER) before performing actions and applying for permits. Please see Appendix 3 for a list of states with Environmental Quality Acts, the specific act to which they are bound, and the governing body of the act.

The Response & Control Working Group works in close association with the TAC, other *Bsal* working groups (e.g., Decision Science, Surveillance & Monitoring, and Clean Trade Working Groups), and partners. In addition, there is continued dialogue with the Research Working Group, Diagnostics Working Group, Outreach & Communication Working Group, Partners in Amphibian and Reptile Conservation (PARC), and the US Geological Survey Research and Monitoring Initiative. For example, the Decision Science Working Group helps biologists decide upon a course of action given the likelihood of success, the Research Working Group focuses on testing possible disease management options, and the Response & Control Working Group assists biologists with implementing management strategies.

**Goal B.1.1:** Review and update the Rapid Response Plan as new information becomes available.

Rank: High

Rationale: As knowledge accrues and response plans are trialed in workshop exercises or in real field or captive situations abroad or in North America, new information or lessons learned may be applied to adapt and improve the Rapid Response Plan.

Management Relevance: A variety of agencies, institutions, and authorities, as well as the general public, may be stakeholders in a *Bsal* response. An efficient, proactively created response plan can aid rapid action if *Bsal* were to be detected. Adapting current plans as new information becomes available elevates plan efficacy and can streamline processes and reduce uncertainty among management partners.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel.

*Priority B.1.1.1:* Stay current with new information that may be used during response actions if *Bsal* were to be detected in North America, including decision science systems, new research on *Bsal* mitigations, results of *Bsal* responses in Europe, biosecurity advances, and surveillance results.

*Priority B.1.1.2:* Interact with other relevant *Bsal* working groups to stay abreast of developing scientific or management breakthroughs that may affect *Bsal* response guidance.

*Priority B.1.2.3:* In the event of a *Bsal* detection in North America, offer assistance for the context-specific customization of the Rapid Response Plan using the template developed by the Response & Control Working Group.

**Goal B.1.2:** Facilitate and improve a natural resource agency’s ability to take proactive and reactive actions to prevent occurrence and transmission of *Bsal* in North America.

Rank: High

Rationale: Should *Bsal* invade North America, it is imperative to not only have a selection of effective mitigation options to counter the threat but also understand the steps needed to implement such actions.

Management Relevance: Proactive management and efficient response to outbreaks by natural resource agencies can be hindered by lengthy approval processes and lack of clarity surrounding the necessary steps. Our actions will offer guidance and tools to biologists, enabling them to efficiently implement strategies on the ground and, ultimately, fostering persistence of our native salamander diversity.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel.

*Priority B.1.2.1:* Define a list of proactive and reactive actions and tools available that can be taken by managers to prevent the introduction and spread of *Bsal*.

*Priority B.1.2.2:* Define and outline justifications for management actions, including effectiveness and possible (or lack of) environmental impacts relevant for applying for a US Department of Interior’s categorical exclusion (Cat Ex) approval.

*Priority B.1.2.3:* For each jurisdiction in North America, identify permits that may be needed for effective *Bsal* mitigation in response to *Bsal* detection at a field or captive site.

*Priority B.1.2.4:* Work with relevant government agencies that have regulatory oversight for actions with potential environmental impacts, under different site contexts, in order to develop an expedited plan for a rapid mitigation of *Bsal* occurrence and transmission.

*Priority B.1.2.5:* Work with relevant government agencies that have oversight for procedures to work with species of conservation concern at sites with *Bsal* detections, including their capture, *Bsal* testing, quarantine in captive settings, treatment, relocation, or euthanization.

*Priority B.1.2.6:* Develop “blanket documents” for exemption or permitting requests, as feasible per jurisdiction and context.

*Priority B.1.2.7:* Identify a list of contacts for rapid submission to relevant permitting agencies and develop a communication chain for expediting processes.

*Priority B.1.2.8:* Explore mechanisms to set up an emergency response fund and determine how to disperse such funds.

**Goal B.1.3:** Provide information and build understanding of *Bsal*, the *Bsal* Task Force, the Strategic Plan, and available management/mitigation options for federal agencies at the national and regional levels.

Rank: High

Rationale: Local and regional management will likely be the first to know of any potential *Bsal* detection and, to be proactive, should be kept well informed; keeping all levels of management informed will help to expedite any processes.

Management Relevance: Local, regional, and national managers can only effectively respond if they are provided with the needed information and understand the possible actions and steps needed to implement actions.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel. External funding resources may be needed for hard-copy brochure production.

*Priority B.1.3.1:* Develop briefing materials about *Bsal*, the *Bsal* Task Force and Strategic Plan, and available *Bsal* management tools (in collaboration with TAC and the Outreach & Communication Working Group).

*Priority B.1.3.2:* Develop a preliminary list of key national (in collaboration with the TAC) and regional (in collaboration with the national Disease Task Team of Partners in Amphibian and Reptile Conservation, as they have this list for their Herpetofaunal Disease Alert System) wildlife health and natural resource contacts for briefing material distribution.

*Priority B.1.3.3:* Devise a plan for briefing material distribution at national and regional scales.

*Priority B.1.3.4:* Distribute a *Bsal* informational brochure/white paper to local field offices of federal, state, tribal, and local agencies that may have vested interest in the detection and mitigation of *Bsal*.

**Goal B.1.4:** Brief and offer training to natural resource agencies (local to regional) about the North American *Bsal* Task Force and available management/mitigation options.

Rank: High

Rationale: Local and regional management will be the first to know of any potential *Bsal* detection and, to be proactive, should be kept well informed. Keeping all levels of management informed will help to expedite any processes.

Management Relevance: Local, regional, and national managers can only effectively respond if they are provided with the needed information and understand the possible actions and steps needed to implement actions.

Financial Needs: In-kind support from member agencies and institutions for personnel time, travel, and printing of training materials. Grants may be needed to cover costs of in-person, on-site standardized training across North America.

*Priority B.1.4.1:* Develop training materials for *Bsal* mitigation actions (in collaboration with the Outreach & Communication Working Group).



*Priority B.1.4.2:* Conduct a mock training session to assess efficacy of the pilot approach developed for the roll-out of management/mitigation options. Revise procedures as warranted.

*Priority B.1.4.3:* Provide training workshops, which could be done in-person or remotely, targeting local, regional, and national natural resource management groups (may vary by region, state/province/territory, or country).



Fire Salamander (*Salamandra salamandra*) infected with *Bsal*, as evidenced by the dark, circular lesions across its body. © Frank Pasmans.

## 2. Diagnostics Working Group

**Objective:** To assist with the promotion of consistent standards among the wildlife health community for detecting *Bsal* and diagnosing *Bsal* chytridiomycosis; to serve as a forum for exchanging ideas, working out challenges, and providing consultation and expert advice concerning *Bsal* detection and chytridiomycosis diagnosis.

**Working Group Lead:** Jacob Kerby (University of South Dakota, Vermillion, SD, USA)

**Past Working Group Lead:** María J. Forzán (College of Veterinary Medicine, Long Island University, NY, USA)

**Background:** Effective detection of novel disease-causing pathogens, such as *Bsal*, relies both on gross and microscopic (histopathologic) examination of lesions in affected animals and on the detection and correct identification of the pathogen through histochemical and molecular methods. Molecular detection of pathogens is a rapidly developing discipline largely reliant on technological advances in analyzing organismal DNA and RNA. Mortality due to *Bsal* was first described by Martel et al. (2013), who also provided a morphological description of the pathogen and the histopathologic lesions it caused and developed a polymerase chain reaction (PCR) assay specific to the 5.8S rRNA gene of *Bsal* that could be run on skin samples. As *Bsal* is closely related to *Batrachochytrium dendrobatidis* (*Bd*), and since bi-pathogen infections are possible, Blooi et al. (2013) developed a duplex real-time PCR assay to detect and differentiate both pathogens. Further advancing the development of reliable standardized diagnostic procedures for *Bsal* is an overarching goal for the working group.

The *Bsal* Diagnostics Working Group was convened as a partnership among diagnosticians and researchers interested in further development of effective diagnostics tools for *Bsal*, addressing Framework Action 3 (Box A5.1). The Diagnostics Working Group is composed of professionals with expertise in the application and interpretation of an array of diagnostic tools, with members working in academia, diagnostic laboratories, and government agencies

throughout North America and involved in detection and reporting of amphibian diseases, including *Bsal*.

Since the working group's assembly in 2015, collaborations between members of the group and others have achieved several initial goals. First, the *Bsal* case definition (White et al. 2016) was published to establish criteria to promote standardized communication of diagnostic results for diagnosis of *Bsal*-caused disease, i.e., *Bsal* chytridiomycosis. The case definition describes the clinical and histopathological presentation of *Bsal* chytridiomycosis and references Martel et al. (2013) and Blooi et al. (2013) for molecular analyses by PCR. Since then, additional recommendations on diagnosing *Bsal* chytridiomycosis have been produced (see Thomas et al. 2018). A second achievement was the completion of a pilot multiple-laboratory round-robin proficiency test for *Bsal* detection by PCR in 2016 and the development of a methodology and logistics plan for a full round-robin, both with funding from Environment and Climate Change Canada. Third, an in situ hybridization protocol to detect *Bd* and *Bsal* cells in formalin-fixed paraffin-embedded tissues was developed (Ossiboff et al. 2019)—definitive differentiation of *Bd* and *Bsal* in tissue sections of affected amphibians is impossible based on fungal morphology and routine histologic stains alone. As the case definitions for *Bsal* and *Bd* chytridiomycosis require both histologic and molecular evidence of infection, this new test to simultaneously screen for and differentiate the two fungal pathogens in tissue sections is critical for accurate diagnosis. Ongoing work by the group is capitalizing upon the value of collaborations among diagnosticians and researchers with different expertise. Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

The Diagnostics Working Group has close interactions with the Research Working Group and the Surveillance & Monitoring Working Group and has intermittent interactions with the Response & Control, Data Management, and Outreach & Communication Working Groups.

**Goal B.2.1:** Establish a long-term program for inter-laboratory quality control and evaluation of protocols for the detection of wildlife pathogens, particularly *Bd* and *Bsal*.

Rank: Urgent

Rationale: Research and diagnostic laboratories throughout the world run PCR tests to detect wildlife pathogens. Standardization of methodologies is difficult, and it is even more difficult for small research laboratories to acquire a certification granted by organizations such as the American Association of Veterinary Laboratory Diagnosticians or ISO (International Organization for Standardization) committees. An option should exist to provide an accessible method for quality control/quality assurance that will allow participating laboratories to confidentially evaluate the quality of their own results. Based on a successful pilot round-robin proficiency test, a formal program to provide annual quality testing to all volunteer laboratories can be established. The program would provide blind samples to participating laboratories, collate results, and provide feedback to all participants. Two things are crucial to the success of such a program: 1) providing the blind samples free of charge so laboratories with limited budgets are not excluded and 2) maintaining the origin of results confidential so all participants can see where their results compare to the group, but no one is able to match a set of results to a specific laboratory.

**Management Relevance:** Establishing a free and accessible quality control source for laboratories testing for the presence of *Bsal* enhances the overall accuracy of results; more reliable results translate into decreased uncertainty and increase the confidence natural resource managers can place on those results. Reliable results are key when managers are tasked with making decisions for rapid-response actions, should *Bsal* be detected in North America.

**Financial Needs:** Cost estimate: 60,000 USD; partial funding provided by Environment and Climate Change Canada, who covered the development of a methodology and logistics plan.

*Priority B.2.1.1:* Determine a laboratory that can produce annual sets of samples containing pre-determined concentrations of inactivated *Bd* and *Bsal* zoospores in solution.

*Priority B.2.1.2:* Identify a group of laboratories willing to participate in testing blind samples and committed to reporting their results within a pre-determined period of time and following an established format that includes a minimum of methodological information.

*Priority B.2.1.3:* Develop a web-based platform for the collection of results and feedback to participating laboratories as well as a deposit of information regarding recommended methodologies.

*Priority B.2.1.4:* Provide a set of blind samples that includes blanks and one or both amphibian chytrid fungi (*Bsal* and *Bd*).

*Priority B.2.1.5:* Collate reports from participating laboratories and provide feedback to all participants. Produce a list of participating laboratories to share with agencies and other institutions interested in submitting samples for testing or collaborating in testing projects.

**Goal B.2.2:** Develop standardized and reproducible methods that will allow comparison across studies, with reliable detection of *Bd* and *Bsal* and reliable estimation of infection load once either or both pathogens are detected.

**Rank:** Urgent

**Rationale:** Numerous laboratories are already running *Bsal* PCR tests, both in native and exotic amphibians. Testing various protocols and establishing one that is most effective and that can fit the majority of technical settings would empower laboratories and provide an easier way to compare results among them. A common request from diagnosticians and researchers is the establishing of a set of recommended standards.

**Management Relevance:** Development of efficient and standardized diagnostic procedures allows comparison of results among contexts, such as different sites. This approach provides a foundation for geographic or taxonomic comparisons of *Bsal* detection and reduces uncertainty, allowing natural resource managers to more confidently implement rapid response actions should *Bsal* be detected in North America.

**Financial Needs:** 135,000 USD; unfunded at present.

*Priority B.2.2.1:* Establish a short list of protocols that are most likely to be used across agencies and institutions.

*Priority B.2.2.2:* Identify a group of laboratories willing to participate in testing blind samples following specific protocols—a subset of the round-robin participants would be best.

*Priority B.2.2.3:* Provide detailed instructions on the protocols to be used for the tests run by participating laboratories.

*Priority B.2.2.4:* Define common metrics that laboratories should report to determine chytrid detection and quantification and the variability of chytrid detection when using molecular tools.

*Priority B.2.2.5:* Provide a set of blind samples that includes blanks and one or both amphibian chytrid fungi (*Bsal* and *Bd*).

*Priority B.2.2.6:* Collate reports from participating laboratories, establish the protocol(s) that yielded most consistent results, and determine intra-laboratory repeatability and inter-laboratory reproducibility.

*Priority B.2.2.7:* Establish a mechanism to provide laboratories with the standard(s) deemed most appropriate based on the round-robin results.



Flatwoods Salamander (*Ambystoma cingulatum*). © Mark Mandica.

### 3. Research Working Group

**Objective:** To facilitate communication and collaboration among scientists studying *Bsal* in North America and to ensure that high-quality research on *Bsal* is produced rapidly.

**Working Group Lead:** Molly Bletz (University of Massachusetts, MA, USA) and Jonah Piovia Scott (Washington State University, WA, USA)

**Past Working Group Leads:** Doug Woodhams (University of Massachusetts, MA, USA); Matt Gray (University of Tennessee, TN, USA); Reid Harris (formerly of James Madison University and Amphibian Survival Alliance, VA, USA)

**Background:** The breadth of *Bsal* research needs span basic, applied, and theoretical science disciplines; advances in all three arenas are needed to build foundational knowledge essential for responding to *Bsal* emergence. Scientific studies across these arenas broadly address Framework Actions 4 through 6 (Box A5.1). To achieve a broad scope of work, the *Bsal* Research Working Group is a collaboration of scientists with a diversity of expertise from multiple disciplines, including molecular and cellular biology, immunology, ecology, mathematics, pathology, and social sciences. The group strives for inclusion and productive collaboration and to maintain or expand its participants to effectively address open research questions critical to our ability to respond to and manage *Bsal*. The working group is currently composed of >30 members, representing >20 organizations.

The Research Working Group maintains a list of research needs based on recent publications, our current state of knowledge, and key knowledge gaps. The working group ranks research

needs on the list as Urgent, High, or Medium priority. Within each of these categories, research needs are considered to have equal importance. Each year, the list is updated as more information becomes available and ranks change. Ranks are associated with the thematic goals for the Research Working Group, listed below. The research goals identified below represent a comprehensive approach to advancing the understanding of *Bsal*'s potential impact on amphibian host communities and effective response and management approaches should *Bsal* be introduced into North America. The goals are broken down into priority research studies (i.e., action items); achievements are listed in annual reports ([salamanderfungus.org](http://salamanderfungus.org)).

The Research Working Group interacts with several other working groups (Fig. A5.1), including key ties to the Decision Science, Data Management, and Diagnostics Working Groups and additional interactions on specific topics with the Response & Control, Outreach & Communication, and Clean Trade Working Groups. In particular, research can inform management decisions and can evaluate effectiveness of intervention strategies collectively identified in collaboration with the Decision Science Working Group and the Response & Control Working Group. The Research Working Group endeavors to reduce uncertainties that impede proactive and responsive strategies. The Decision Science Working Group will consider multiple objectives, preferences and values of individual decision-makers, risk profiles, current research frontiers, and uncertainty. For example, identifying possible management interventions for infected habitats (Goals B.3.4, B.3.7) can be done in a decision analysis framework to identify the optimal strategy, given species-specific susceptibility (Goal B.3.3), and calculate the importance of reducing remaining uncertainties to improve decisions. Hence, it is essential that the Research Working Group interacts with the other working groups to produce research with applied implications.

**Goal B.3.1:** Understand the role of human behavior and the pet trade in the spread and spillover of *Bsal*.

Rank: Urgent.

Rationale: Past experiences with emerging infectious diseases in wildlife populations have shown that pre-emptive and precautionary actions are essential to the success of containing and reducing the spread of novel pathogens (Langwig et al. 2012). Thus, the investment in *Bsal* research will provide an excellent case example of the usefulness of science-based preparedness in responding to novel pathogen introductions in the wild and is specifically relevant to *Bsal* transmission routes in trade markets.

One of the most likely routes of entry for *Bsal* into North America is unclean international trade of amphibians (Gray et al. 2015). Currently, animal health certificates for internationally traded wildlife are not required for most nations, including the United States. Although the US Fish and Wildlife Service rule banning the trade of some salamander genera may have reduced the likelihood of infected animals entering the United States via trade (Grant et al. 2017), the ban does not include many taxa (e.g., frogs) that are known suitable hosts (based on recent studies or unpublished data). Yuan et al. (2018) estimated that up to 66,000 salamanders infected with *Bsal* could have entered the United States in the past 10 years, and their estimates did not include frogs, which constitute 94% of imported amphibians. Indeed, *Bsal* has been documented in trade in Europe (Nguyen et al. 2017, Fitzpatrick et al. 2018, Sabino-Pinto et al. 2018), and trade is hypothesized as the route of entry from Asia, where *Bsal* is endemic, to the European continent (Martel et al. 2014, Nguyen et al. 2017). Information on the occurrence of *Bsal* in the North

American amphibian pet trade is needed. Klocke et al. (2017) performed preliminary surveillance for *Bsal* in the United States pet trade and did not detect it. However, their small sample size may have prevented detection of the pathogen at low prevalence (Yuan et al. 2018). Limited *Bsal* surveillance studies in the pet trade have been published for Canada and Mexico (Govindarajulu et al. 2017). In the case of Mexico, a formal petition has been made to the corresponding federal agency (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria [SENASICA]) so that amphibian imports will be screened for *Bsal*; however, the petition is still under evaluation. Additional information about the potential for commonly traded amphibian species to carry *Bsal* is also needed and will help guide surveillance efforts.

In addition to knowing whether *Bsal* exists in North America and its prevalence in the continent's pet trade, we need to understand human dimensions, e.g., the likelihood of consumers releasing unwanted pet amphibians or disposing of their aquarium contents in the environment. This likelihood may differ between hobbyist and specialist amphibian consumer groups. If *Bsal* is detected in the pet trade or the wild, it is important to know the willingness of consumers to participate in programs designed to modify public behavior in a way that will limit pathogen spread, such as providing unwanted pet amphibian amnesty programs and using disinfectants known to kill *Bsal* zoospores at home (aquaria) or in the field (recreational gear).

The studies described below will use a combination of non-lethal testing of amphibians in the pet trade for *Bsal* infection, methods to understand carrier capacity of highly traded amphibians, and human dimension surveys to characterize public awareness, perceptions, and behaviors associated with *Bsal*.

Management Relevance: The North American Model of Wildlife Management relies on evidence-based decision making. For an effective *Bsal* management response, science-based support for context-specific pathogen occurrence, host disease susceptibility, and efficacy of management or treatment alternatives is imperative to reduce uncertainty in manager or policy-maker decision making. Surveillance for *Bsal* in the pet trade is essential to knowing whether this foreign pathogen is in North America. Estimates of prevalence can be combined with shipping and distributor information to identify areas where spillover is most likely to occur, which can direct field activities. Understanding human behavior is also essential to assessing risk of human-mediated spillover or translocation of *Bsal* among sites and estimating public perceptions of future programs or regulations designed to thwart *Bsal* emergence.

Financial Needs: 30,000 USD per state/province/territory/port of entry; 15,000 USD per species; partial funding received for species research. A study by Klocke et al. (2017) addressed Priority B.3.1.1.

*Priority B.3.1.1:* Estimate the occurrence and prevalence of *Bsal* in the North American pet trade through non-lethal surveillance of amphibians at ports of entry, wholesale distributors, and retail stores.

*Priority B.3.1.2:* Estimate the susceptibility of potential *Bsal* hosts (salamanders and frogs) in the pet trade (e.g., species commonly imported from Southeast Asia).

*Priority B.3.1.3:* Characterize human behaviors for amphibian hobbyist and specialist groups to estimate the likelihood of *Bsal* spillover from consumers to wild populations and the acceptance of public outreach strategies designed to limit the anthropogenic spread of *Bsal*.



**Goal B.3.2:** Identify critical transmission pathways and conditions under which *Bsal* is likely to emerge in amphibian host populations in North America (e.g., compartmental disease models).

Rank: Urgent.

Rationale: Identifying the importance of transmission pathways under varying conditions is fundamental to characterizing the epidemiology of host–pathogen systems and developing effective disease intervention strategies (Tien and Earn 2010, Langwig et al. 2012). Environmental transmission of *Bsal* can occur through water or soil, and it depends on various factors, such as host shedding rates of the pathogen and pathogen persistence outside of the host (Nelson et al. 2009, Briggs et al. 2010, Stegen et al. 2017). Transmission can also occur through direct contact between infected and uninfected individuals. The probability of transmission can change as disease progresses in the host (McCallum et al. 2001, Peace et al. 2019). We recommend development of *Bsal* epidemiology models for widely distributed, abundant host species in North America that are known to be susceptible to *Bsal* (e.g., Eastern Newt [*Notophthalmus viridescens*], Rough-skinned Newt [*Taricha granulosa*]), given their potential to maintain, amplify, and spread *Bsal*. The action items below outline the parameterization of models that can be used to identify key transmission pathways and conditions under which *Bsal* is likely to emerge. The proposed work involves a combination of controlled experiments and mathematical modeling following models developed for *Bd* (Briggs et al. 2010), *Ranavirus* spp. (Peace et al. 2019), and *Bsal* (Schmidt et al. 2017).

Management Relevance: These predictive models can provide insight into transmission pathways, environmental conditions, and population characteristics that can be manipulated to reduce the impacts and persistence of *Bsal* at a site. For example, if direct contact between individuals is a key transmission pathway, intervention strategies that reduce contacts should be used, such as altering habitat structure or reducing animal density. If environmental transmission is a key pathway, strategies that change conditions to reduce zoospore persistence should be implemented. If non-amphibian hosts can contribute to the persistence of *Bsal* in the environment, strategies can be directed at managing these groups.

Financial Needs: 2.1 million USD; partial funding by the United States’ National Science Foundation for Priorities B.3.2.1–B.3.2.5. A study by Longo et al. (2019) addressed Priority 3.2.6.

*Priority B.3.2.1:* Estimate latency period of infection and recovery rate for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.

*Priority B.3.2.2:* Estimate daily shedding and encystment rate of *Bsal* zoospores and the infectious dose (ID)<sub>50</sub> for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.

*Priority B.3.2.3:* Estimate daily contact rates of amphibian hosts at relevant temperatures and densities when exposed to different complexities of habitat structure.

*Priority B.3.2.4:* Estimate probability of *Bsal* transmission between infected and uninfected amphibian hosts (within and between species) at different post-exposure durations and temperatures.

*Priority B.3.2.5:* Estimate the duration of zoospore persistence in water and soil given differences in various environmental conditions (e.g., temperature, micropredators, soil moisture, water chemistry, and bacterial presence).

*Priority B.3.2.6:* Estimate the influence of co-infection with other pathogens (e.g., *Bd*, *Ranavirus* spp.) on the likelihood of *Bsal* transmission and development of chytridiomycosis.

*Priority B.3.2.7:* Determine the probability of transmission or translocation of *Bsal* by non-amphibian hosts (e.g., crayfish, waterfowl, humans) and understand their role as potential biological reservoirs.

**Goal B.3.3:** Produce more informed *Bsal* risk models for North America through improved, objective classification of species susceptibility and tolerance to *Bsal* infection (e.g., integral projection models).

Priority: Urgent

Rationale: The likelihood of pathogen invasion is commonly modeled using risk analyses, which can be dependent on environmental conditions, host species distribution and susceptibility, and population characteristics (Václavík et al. 2010, OIE and IUCN 2014). Preliminary *Bsal* risk models for North America based on environmental suitability indices for *Bsal* and salamander distributions suggest that the Southeast, Northeast, and Pacific Coast of the United States and south-central Mexico have high invasion potential (Yap et al. 2015, 2017; Richgels et al. 2016; Basanta et al. 2019). One limitation of these predictions is that little information was available for incorporating host susceptibility into the risk estimates. Evidence for species susceptibility is being rapidly expanded (Martel et al. 2014, Barnhart et al. 2020, Carter et al. 2020; unpubl. data). Since autumn 2015, the susceptibility of a number of North American amphibian species to *Bsal* has been estimated among several United States laboratories (Appendix 1). Integral projection models (IPMs) can be used to categorize species susceptibility, considering their tolerance to infection (Wilber et al. 2016). Susceptibility indices can be combined with host species distributions and environmental niche data for *Bsal* to more robustly predict risk of pathogen invasion geographically. Biologists can use risk assessments to target locations for disease response and management actions. IPMs can also be used to classify the potential role of species during disease outbreaks (Wilber et al. 2016), which could range from resistant to reservoir to amplification hosts (Paull et al. 2012). Knowing the potential contribution of host species to community-level transmission can help direct disease intervention strategies, which can differ depending on host susceptibility (Streicker et al. 2013). The proposed work involves a combination of dose-dependent experiments and mathematical modeling to objectively categorize and rank species susceptibility.

Management Relevance: Comprehensive assessment of species susceptibility to *Bsal* in North America will produce robust *Bsal* risk maps (similar to Yap et al. 2015, Richgels et al. 2016, Basanta et al. 2019) highlighting areas in which pathogen surveillance and disease response actions can be targeted. Additionally, IPMs can lead to objective rankings of species susceptibility and classifications of epidemiological roles (e.g., resistant, reservoir or amplification species), which provide insight into community-level impacts at sites. For example, communities dominated by carrier species (i.e., high *Bsal* tolerance) may experience

minimal disease occurrence but have high *Bsal* infection prevalence and be sites where the pathogen is maintained. In contrast, sites dominated by amplification species (i.e., low *Bsal* tolerance) may experience rapid *Bsal* transmission, disease progression, and population declines.

Financial Needs: 15,000 USD per species, Priority B.3.3.1, partially funded by the BAND Foundation; 300,000 USD each, Priority B.3.3.2 (partially funded by the Smith Conservation Fellowship and National Science Foundation) and Priority B.3.3.3 (partially funded by the National Science Foundation); 200,000 USD, Priority B.3.3.4, funded by US Fish and Wildlife Service.

*Priority B.3.3.1*: Estimate the susceptibility (i.e., tolerance) of North American amphibians to *Bsal* infection and chytridiomycosis using standardized, dose-dependent experiments (suggestions for targeted taxa can be provided by the lead of the Research Working Group).

*Priority B.3.3.2*: Estimate the impact of habitat characteristics (temperature, pH, salinity, zooplankton abundance, etc.) on *Bsal* infection risk.

*Priority B.3.3.3*: Develop integral projection models (IPMs) that predict tolerance using temporal estimates of *Bsal* infection load and host fitness metrics (e.g., survival, disease ranking based on microscopic and gross lesions).

*Priority B.3.3.4*: Use information developed in Priorities B.3.3.1–B.3.3.3 to map susceptibility indices on the geographic distributions of hosts and environmental suitability niches for *Bsal* to produce robust spatial predictions of *Bsal* risk in North America.

**Goal B.3.4**: Identify effective methods for managing *Bsal*-induced disease and clearing *Bsal* infections in captive and field settings.

Rank: Urgent

Rationale: Managing disease threats, like those posed by *Bsal*, are of the utmost importance for conservation. A proactive strategy for developing disease mitigation tools is imperative for having an effective rapid response if *Bsal* is introduced into North America (Grant et al. 2017). Priority for disease mitigation should focus on highly susceptible amphibian taxa as well as tolerant hosts that may act as *Bsal* reservoirs within the ecosystem. Mitigation strategies targeting the host, such as vaccination or probiotic bioaugmentation of the skin microbiota, or strategies targeting the environment, such as micropredator augmentation, are possible conservation frontiers for field-based mitigation (Bletz et al. 2013, Garner et al. 2016, Thomas et al. 2019). We can use what we have learned from *Bd* as a foundation for developing and understanding potential disease mitigation and treatment strategies and also take advantage of novel directions. Within the amphibian *Bd* studies, the addition of locally occurring protective bacteria to amphibian skin has effectively prevented *Bd*-associated chytridiomycosis in laboratory trials and a field trial (Harris et al. 2009a,b; Vredenburg et al. 2011). Additionally, early studies suggest that adaptive immunity can be induced by a vaccination strategy (McMahon et al. 2014). Nasal delivery of vaccines against bacterial and viral infectious diseases has shown promising results in Rainbow Trout (*Oncorhynchus mykiss*; La Patra et al. 2015) and may be an effective strategy for treating amphibian species. Furthermore, *Bd* infection risk has

been correlated with environmental micropredators, and certain microeukaryotes can greatly reduce infection probability and reduce zoospore persistence in experimental contexts (Schmeller et al. 2014). Therefore, manipulation of micropredator communities could serve as a feasible strategy to minimize *Bsal* infection risk. A recent review by Thomas et al. (2019) described possible strategies for mitigating *Bsal*, critical knowledge gaps, and future research directions.

Development of infection clearance strategies for traded amphibian species that can carry *Bsal* can allow trade to continue while minimizing the risk of *Bsal* introduction. Heat therapy and antifungal treatments have been found to be effective for European Fire Salamanders (*Salamandra salamandra*) (Bloom et al. 2015a,b). However, such treatments may not be suited for all amphibian species. Many species cannot tolerate elevated temperature and/or antifungal medications (e.g., itraconazole; Baitchman and Pessier 2013).

Management Relevance: Disease response is essential to thwarting pathogen outbreaks. Because amphibians have relatively low dispersal capability, host- and site-based management strategies can be effective, and their effectiveness has been demonstrated in some cases for *Bd* (Vredenburg et al. 2011, Bosch et al. 2015). Upon identification of effective strategies, natural resource agencies will be equipped with the best practices to prevent (proactive) or reduce (reactive) *Bsal* chytridiomycosis in amphibian habitats and populations.

Financial Needs: 900,000 USD total, Priorities B.3.4.1–B.3.4.6; 150,000 USD per strategy, partially funded by the David H. Smith Conservation Fellowship, Foundation for the Conservation of Salamanders, US Fish and Wildlife Service; 600,000 USD total, Priorities B.3.4.7–B.3.4.11, partially funded by the National Science Foundation and US Fish and Wildlife Service.

*Priority B.3.4.1:* Identify effective probiotic microbes and develop probiotic treatment methods to combat *Bsal*, including the exploration of host and environmental modes of treatment. Test non-target impacts of probiotics and examine potential for bacteremia through lesions.

*Priority B.3.4.2:* Identify *Bsal*-consuming aquatic micropredators from natural habitats and test micropredator augmentation strategies.

*Priority B.3.4.3:* Evaluate novel vaccination methods as a possible disease mitigation tool and test different modes of delivery (e.g., different life stages, nasal-associated lymphoid tissue vaccination, skin exposure, nanoparticle technology).

*Priority B.3.4.4:* Explore the use of *Bsal* removal methods (e.g., attractants or traps).

*Priority B.3.4.5:* Explore the genetic correlates of disease resistance and the possibility of selectively breeding hosts for *Bsal* resistance.

*Priority B.3.4.6:* Evaluate the potential use of disinfectants in the field to eradicate *Bsal* from a small area after a point source introduction (sensu Bosch et al. 2015).

*Priority B.3.4.7:* Determine minimal alterations to habitats that can promote disease risk reductions (e.g., increasing habitat temperature through shade reduction, altering pH or salinity, changing complexity of habitat structure to affect contact rates, dewatering habitats) or augment habitats with native anti-*Bsal* microbes.

*Priority B.3.4.8:* Determine the effectiveness of reducing host density or altering relative abundance of host species with different infection tolerances on the invasion potential of *Bsal*.

*Priority B.3.4.9:* Identify volatile organic compound (VOC)-producing, *Bsal*-inhibitory microbes and their inhibitory compounds.

*Priority B.3.4.10:* Test alternative antifungal compounds for use on a broad host taxonomic range and across life-history stages.

*Priority B.3.4.11:* Test the use of microbes and/or compounds to clear existing infections and minimize side effects.

**Goal 5:** Quantify innate and adaptive immune responses to *Bsal* across species and environmental conditions.

Rank: High

Rationale: Little is known about the immune defenses of salamanders against *Batrachochytrium* fungi. Preliminary research suggests that the Fire Salamander (*Salamandra salamandra*)—a European newt species in which exposure to a low dose of *Bsal* results in disease—has few effective immune defenses against *Bsal* infection (Martel et al. 2013, Van Rooij et al. 2015). Other salamander species appear to be more resistant to *Bsal* chytridiomycosis, and several anuran species can clear infection (Martel et al. 2014, Stegen et al. 2017). Despite initial findings with species susceptibility trends (Goal B.3.3), the role of amphibian immune defenses in mediating host response to *Bsal* infection remains largely unknown. Immunocompetence in amphibians can differ among life-history stages (i.e., age classes), among populations, and with changes in environmental conditions. In particular, amphibian immunity is influenced by temperature (Rollins-Smith 2017). Like other vertebrates, the immune system of amphibians includes innate and adaptive components. For skin pathogens like chytrid fungi, antimicrobial peptides produced in the skin can be an important first defense (Holden et al. 2015). Symbiotic microorganisms on amphibian skin can also contribute to immunity through direct competitive interactions or by producing antimicrobial byproducts (Woodhams et al. 2018). Adaptive immune responses to *Bsal* are unknown. Understanding the mechanisms of host disease resistance can lead to the development of intervention strategies focused on host immunity, such as use of vaccines and bioaugmentation techniques. Because *Bsal* creates necrotic skin ulcerations that can extend through the epidermis (Martel et al. 2013), possible probiotic treatments need to be evaluated to ensure they do not contribute to bacteremia and sepsis (Bletz et al. 2018).

Management Relevance: A mechanistic understanding of amphibian immune responses to *Bsal* will enable directed mitigation approaches. For example, determining how protective immunity can be established in salamanders may direct management toward vaccines or probiotic microbial therapy or other approaches to increase salamander resistance to chytridiomycosis. It also may be possible to alter habitat conditions to facilitate some host immune responses.

Financial Needs: 1.3 million USD, partial funding from the National Science Foundation, Eastern Newt focus.

*Priority B.3.5.1:* Determine whether amphibians are able to develop a lymphocyte-mediated immune response to *Bsal* and how this and other responses compare among species, populations, and life stages and across environmental conditions.

*Priority B.3.5.2:* Determine whether salamanders produce antimicrobial skin peptides or other antimicrobial compounds and if skin toxins used for defense (e.g., tetrodotoxin, TTX) influence antimicrobial product production.

*Priority B.3.5.3:* Determine whether amphibians or symbionts produce antifungal small molecule compounds.

*Priority B.3.5.4:* Determine how the skin microbiome (bacteria, fungi, viruses) interacts with immune responses and influences disease susceptibility. Also, determine if the skin microbiome can be manipulated and whether it varies with environmental conditions and host genetics.

*Priority B.3.5.5:* Establish hematological reference values and determine how these parameters reflect immunity to *Bsal* infection in amphibian hosts.

*Priority B.3.5.6:* Determine whether there is protective immunity that develops upon host clearance of *Bsal* and repeat exposure. Determine how protective immunity can best be established (e.g., vaccine, heat-clearing *Bsal*). Also, determine what immune responses are regulated by protective immunity (e.g., mucosal antibodies, skin defense compound expression, changes in microbiome).

**Goal B.3.6:** Identify the mechanisms of *Bsal* pathogenesis.

Rank: High

Rationale: The mechanisms by which *Bsal* becomes a lethal pathogen are unknown (Van Rooij et al. 2015). Grossly and anatomically, chytridiomycosis due to *Bsal* develops differently in a host than does *Bd*. *Bd* results in hyperkeratosis (i.e., skin thickening), whereas *Bsal* causes ulcerative, necrotic skin lesions that can extend through the epidermis. The mechanisms of pathogenesis for *Bd* are compromised osmoregulation across the skin that leads to electrolyte imbalance in the blood (especially  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ ), which affects epidermal electrolyte transport, leading to asystolic cardiac arrest (Voyles et al. 2009). The physiological mechanisms for *Bsal* pathogenesis may be similar to *Bd* (i.e., altered osmoregulation); however, electrolyte imbalance may be a consequence of skin destruction instead of hyperplasia. It is also possible that reduced cutaneous respiration could be a morbidity factor in *Bsal*-induced chytridiomycosis. In general, salamanders rely on cutaneous respiration more than frogs, especially species in the Plethodontidae (lungless salamander) family (Wells 2007). Bacteremia is another hypothesized mechanism of *Bsal* chytridiomycosis (Bletz et al. 2018).

The proposed work involves a combination of clinical and anatomical pathology to quantify structural and physiological changes in salamanders as *Bsal* chytridiomycosis progresses. Additional areas of exploration will include the molecular pathways required for initial interactions between *Bsal* zoospores and their hosts. In particular, understanding how the *Bsal* zoospore is attracted to a suitable host (e.g., chemotaxis) and adheres are important areas for research and represent possible opportunities for prevention or reduction of infection. Other areas of research focusing on *Bsal* biology that will provide important insight into pathogenesis

include understanding how *Bsal* infection spreads through host tissue and identifying molecular signatures specific to host infection.

**Management Relevance:** Understanding the pathology of *Bsal* will enhance our ability to predict susceptible species and provide the groundwork for making informed decisions about where and how to manage *Bsal* emergence.

**Financial Needs:** 1.2 million USD; partially funded by the National Science Foundation.

*Priority B.3.6.1:* Quantify the changes in plasma electrolyte concentrations in *Bsal*-infected salamanders.

*Priority B.3.6.2:* Identify whether bacterial invasion through the skin via *Bsal* lesions and sepsis are contributing factors to pathogenesis.

*Priority B.3.6.3:* Identify tissue tropism for *Bsal*-infected amphibians.

*Priority B.3.6.4:* Explore mechanisms of attraction to (chemotaxis) and physical binding of zoospores to hosts.

*Priority B.3.6.5:* Determine whether *Bsal* releases lymphotoxic or cytotoxic molecules.

**Goal B.3.7:** Establish effective methods for detecting *Bsal* infections.

**Rank:** Urgent

**Rationale:** Managing disease threats, like those posed by *Bsal*, are of the utmost importance for conservation. A first step is preventing entry of the pathogen into naïve regions like North America, and therefore, rapid and accurate detection of the pathogen is crucial. Recent histological advances have provided a novel tool for identifying coinfections using RNAScope (Ossiboff et al. 2019). This objective intersects directly with the Diagnostics Working Group.

**Management Relevance:** Detecting and tracking existing *Bsal* infections from captive-housed amphibians is critical in the pet trade and for captive management of critically endangered amphibians or amphibians used in research. Improved methods may enable more effective policy recommendations and make it easier to eliminate threats.

**Financial Needs:** 350,000 USD; unfunded at present.

*Priority B.3.7.1:* Develop new diagnostic tools and improve existing tools.

**Goal B.3.8:** Estimate the interactive effects of *Bsal* with natural and anthropogenic stressors.

**Rank:** Medium

**Rationale:** Laboratory estimations of the susceptibility of amphibian species to *Bsal* are a good starting point for developing landscape risk models for *Bsal* emergence. However, amphibians have complex life histories and unique physiologies that make them particularly sensitive to stressors. Indeed, amphibians are heavily dependent on water, making them particularly sensitive to altered hydroperiod, desiccation, and decreases in water quality. Examples of impaired water

quality include increased salinity, acidity, eutrophication, and pesticide contamination. In many cases, environmental stressors induce changes in host behavior and physiology that could potentially influence risk from *Bsal*. For example, changes in body condition or corticosterone (a hormone commonly elevated in response to stressors) can modulate immune function and possibly susceptibility to *Bd* (Tatiersky et al. 2015, Fonner et al. 2017). Similarly, physiological and behavioral responses to desiccation (e.g., changes in plasma osmolality, increased osmoregulatory behaviors) may influence infection dynamics and disease progression. These effects may be exacerbated or mitigated in more complex environments (e.g., mesocosms) by changes in community interactions and habitat quality.

Management Relevance: Understanding how environmental and community conditions modulate susceptibility to *Bsal* will help predict invasion risk. In addition, if stressors are identified (e.g., pesticides), management strategies can be implemented to reduce the effect of the stressor.

Financial Needs: 150,000 USD per stressor, Priority B.3.8.1; 300,000 USD Priority B.3.8.2.

*Priority B.3.8.1:* Conduct susceptibility trials that include common natural and anthropogenic stressors (e.g., hydration, salinity, pesticides) to determine if outcomes following *Bsal* exposure are altered.

*Priority B.3.8.2:* Conduct susceptibility trials in complex settings that include community features such as predation and trophic interactions and changing habitat quality.





Northern Gray-cheeked Salamander (*Plethodon montanus*). © Alberto López.

#### 4. Decision Science Working Group

**Objective:** To support management decisions regarding *Bsal* through the facilitation of decision-making processes, identification and collation of information needed to make decisions, development of models to predict the outcomes of different management options, and evaluation of trade-offs and risks to overcome impediments to optimal decision-making.

**Working Group Lead:** Evan Grant (US Geological Survey Patuxent Wildlife Research Center, MA, USA)

**Background:** The Decision Science Working Group applies the theory, tools, and techniques from decision analysis to the complex decision-making process for mitigating the threat of *Bsal*, managing risk to native amphibian communities, and responding to *Bsal* detections in North America (Framework Actions 2 and 5; see Box A5.1). Application of decision science provides an effective interface between rapidly changing conditions in societal, policy, and science information, uncertainty, and operational planning, which together provide substantial benefits that facilitate effective and rapid decision-making in response to *Bsal* detection. The Decision Science Working Group is currently composed of members representing academic institutions and federal agencies (US Geological Survey, US Fish and Wildlife Service). Collectively, the group has decades of experience in decision science, amphibian and pathogen ecology, research, mathematical modeling, and direct work with managers.

Emerging diseases have the potential to affect social, economic, and ecological interests of North American resource managers, who are entrusted by society to manage protected areas and wildlife populations. Because they must consider multiple objectives, resource managers face difficult trade-offs for any disease management strategy. The complexity that arises in balancing numerous, competing demands on resource managers effectively limits our ability to identify and implement proactive management, representing a major challenge for developing management strategies for *Bsal* and other emerging infectious diseases. To date, there are no viable treatment options available for *Bsal*, which limits the alternatives available for managers until effective treatments are identified (the Research Working Group has identified research priorities to address this knowledge gap). Much uncertainty remains, which also makes choosing an

(untested) management action challenging. Decision science provides a framework for developing strategies and determining a course of action in the face of uncertainty. Additionally, even if treatments are identified, implementation may still be delayed if other management objectives are predicted to suffer; decision analysis helps identify optimal solutions across potentially competing management objectives.

Planning for the possible arrival of *Bsal* in North America illustrates several decision-making impediments common in the world of wildlife disease. First, despite calls for improved responses to emerging infectious diseases in wildlife, management is seldom considered until a disease has been detected in a population. Lack of resources (i.e., time, money, and personnel) are often cited as reasons for not taking pre-emptive actions, but reactive approaches often limit the potential for control and increase the total cost of a response. Second, while preventing the arrival of a pathogen is the most effective means of controlling emerging infectious disease, it is not fail-safe. Once present in a new area or population, emerging diseases have the potential to impact competing social, economic, and ecological interests of North American resource managers, and there are consequently difficult trade-offs for any given disease prevention or management strategy (e.g., optimal actions for managing a wildlife disease may result in declines in recreational or economic values). Finally, acting under high levels of uncertainty is a hallmark of wildlife disease management. Choosing an untested management action can be difficult for managers to justify to the public, and acting in the face of uncertainty depends a great deal on an individual manager's tolerance for risk.

The application of decision science is increasing among natural resource agencies, as it provides rational and transparent frameworks for managing disease. Decision science tools, such as cost-benefit analysis or portfolio decision theory, can help managers better understand the opportunity costs of proactive action versus inaction. Multi-criteria decision analysis can be used to help examine trade-offs among competing social, political, economic, and ecological objectives. Finally, decision trees, expected value of information, and Bayesian belief networks are useful tools for understanding risk tolerance and examining the trade-offs between managing despite uncertainty and delaying action to gain additional information. By using the tools from decision science to facilitate conversations between researchers and wildlife managers and identify optimal management strategies, the Decision Science Working Group can help navigate the common pitfalls of developing and implementing proactive management solutions for *Bsal* ahead of an invasion and plan for thoughtful responsive management once *Bsal* arrives in a population.

Since 2015, the Decision Science Working Group has made considerable progress on its initial goals. The inaugural meeting that led to the development of the North American *Bsal* Task Force was originally planned as a *Bsal* decision science workshop sponsored by the US Geological Survey. That workshop led to a report that was one of the *Bsal* Task Force's initial accomplishments (Grant et al. 2015) and helped springboard the first product of the Decision Science Working Group (Grant et al. 2017), which addressed proactive measures to forestall *Bsal* emergence in North America. Simultaneously with the formation of the *Bsal* Task Force, initial efforts of an independent subgroup that became the heart of the Decision Science Working Group came to fruition as their United States risk assessment for *Bsal* emergence was published (Richgels et al. 2016). The Decision Science Working Group's current goals are designed to help researchers and managers identify and address the kinds of decision-making impediments outlined above and to advance the overall Framework Actions of the *Bsal* Task Force (Box A5.1). Goals and priority actions per goal are described further in the *Bsal* Implementation Plan

(Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

Some major challenges to *Bsal* management include limited control options for the initial introduction of disease, widely dispersed populations over multiple states and regions, fragmented management authority by diverse agencies (state or provincial/territorial, federal, and non-profits), and deep uncertainties in ecological characteristics of the pathogen, populations, and effectiveness of potential treatments. The Decision Science Working Group goals below are designed to respond to these challenges, and many are interactive with other working groups, especially the Response & Control, Research, Data Management, and Outreach & Communication Working Groups.

**Goal B.4.1:** Identify critical research that has the highest value of information, which will lead to an improved ability to manage *Bsal*.

Rank: High

Rationale: Identification of critical research needs that impede decision-making is of paramount importance for responsive and proactive management of *Bsal* emergence in North America. The collaborative development of research priorities between land managers and researchers is an integral component for creating and evaluating effective and efficient management solutions.

Management Relevance: Despite calls for improved responses to emerging infectious diseases in wildlife, management is seldom considered until a disease has been detected in a population. Reactive approaches often limit the potential for control and increase the total cost of a response. By using the tools from decision science to facilitate conversations between researchers and wildlife managers and identify optimal management strategies, the Decision Science Working Group can help navigate the common pitfalls of developing and implementing proactive management solutions for *Bsal* ahead of an invasion and plan for thoughtful responsive management once *Bsal* arrives in a population. Acting under high levels of uncertainty is a hallmark of wildlife disease management, and the use of formal decision analytics (e.g., multi-criteria decision analysis, risk analysis, cost–benefit analysis within a structured or adaptive management framework, and portfolio decision theory) is increasing among natural resource agencies as a rational and transparent framework for managing diseases. Decision analytic approaches can examine trade-offs between managing despite uncertainty and delaying action to gain additional disease information. In addition, this framework can identify key trade-offs among competing objectives, which are often ignored but which can be highly influential in the final decision-making process and in optimizing management responses.

Financial Needs: 500,000 USD; unfunded at present.

*Priority B.4.1.1:* Coordinate a *Bsal* science experts workshop to collaboratively create a system diagram to help identify areas of greatest research need (i.e., regions within the system diagram that may facilitate the development of proactive management strategies). System, or influence, diagrams map ecological system components and relationships that lead to defined outcomes. Research priorities are generated for those areas of the system diagram where improved knowledge will have the greatest contribution to selecting

optimal management actions and can be formally assessed using decision-analytic tools. This work will be conducted in collaboration with the Research Working Group.

**Goal B.4.2:** Identify approaches to improve proactive management of *Bsal* when risk or competing objectives are impediments to action.

Rank: High

Rationale: Proactive management to forestall *Bsal* emergence reduces the need for crisis-management approaches that can be less effective, less cost-efficient, and potentially higher risk for values of concern, such as threatened species or personal property.

Management Relevance: Involvement of the natural resource management community in proactive measures to forestall *Bsal* transmission in North America has several strategic advantages: it raises awareness of the potential *Bsal* threat to native species; it increases the likelihood *Bsal* is detected before it gains a significant foothold on the continent; as biosecurity measures are enacted, there are likely “spillover” benefits to forestall transmission of other pathogens, microparasites, or invasive species that similarly affect endemic hosts; it improves management success; and it is cost-effective relative to reactive measures that may include long-term rescue of local endemic hosts at the risk they may become conservation reliant for their persistence.

Financial Needs: 500,000 USD; unfunded at present.

*Priority B.4.2.1:* Use simulation, modeling, and optimization techniques to identify optimal actions given various impediments (i.e., uncertainty or competing objectives).

*Priority B.4.2.2:* Using these models, evaluate possible trade-offs of action versus inaction and estimate costs of delaying action. Evaluate individual and agency risk tolerance and its effect on optimal actions under different levels and sources of uncertainty.

**Goal B.4.3:** Conduct and update *Bsal* risk assessments.

Rank: High

Rationale: Based on recent risk assessments, amphibian importation restrictions were instituted in the United States and Canada in response to the threat of *Bsal* invasion. Unfortunately, the banning of salamander imports is unlikely, by itself, to completely mitigate the risk of introduction and spread (OIE and IUCN 2014) of this disease. For example, restrictions on the movement of domestic birds in 2015 failed to prevent highly pathogenic avian influenza outbreaks, which were attributed to poor or incomplete adherence to biosecurity recommendations. In addition, *Bsal* has recently been detected on several commonly imported anuran species in addition to urodeles, and the complete range of *Bsal* amphibian hosts is unknown. Thus, while the US Fish and Wildlife Service and Environment and Climate Change Canada decisions are an excellent first step to protecting North American salamander species from *Bsal* introduction, here we further explore the effectiveness of the possible combination of

prevention strategies for mitigating risk from an emerging pathogen, using *Bsal* as a case example.

**Management Relevance:** Knowledge advances can be used to inform adaptive management processes, improving management efficacy. This cycle can be applied to decision science and risk assessments, where knowledge advances can be used to update parameters in risk models and to alter management and policy decisions. In the *Bsal* context, use of multiple *Bsal* prevention strategies, rather than reliance on a single measure, can be a more effective strategy to forestall *Bsal* emergence in North America. In addition to an import restriction on salamanders, addition of alternative measures warrant consideration.

**Financial Needs:** 200,000 USD; unfunded at present.

*Priority B.4.3.1:* Estimate the residual risk to populations after implementation of strategies (e.g., importation ban, clean trade certification, or other trade-based strategies) designed to reduce risk of introduction of *Bsal* into wild populations of amphibians in Canada, Mexico, and the United States.

*Priority B.4.3.2:* Identify how other actions, in combination, may further reduce risk to native amphibians. Remaining risk will be calculated for combinations of pre-introduction (proactive) and post-introduction (responsive) management actions.

**Goal B.4.4:** Frame *Bsal* management problems at regional and resource manager levels.

**Rank:** High

**Rationale:** This goal is the bulk of the work needed to plan and develop implementation strategies for management of *Bsal* risk. Several managers are working with the Decision Science Working Group to frame and evaluate their decision options for proactive *Bsal* management. Framing management problems as decisions can enable managers to identify possible proactive solutions. This approach recognizes context-specific constraints, such as agency mandates, trade-offs among other mission elements, and relevant uncertainties that must be accommodated in developing a response.

**Management Relevance:** Having natural resource managers work together with decision science specialists can expedite development of multiple effective management options for *Bsal* response that take diverse management contexts into consideration.

**Financial Needs:** 680,000 USD; unfunded at present.

*Priority B.4.4.1:* Engage resource managers at multiple scales (e.g., single protected area, regional, national) to develop decision frameworks, and specific and relevant measurable attributes, for their particular jurisdictions. Particular emphasis should be made to include objectives and metrics to understand trade-offs among habitats, amphibian populations, and pathogen occurrence and prevalence.

*Priority B.4.4.2:* Hold a series of structured decision-making workshops with managers (i.e., US Fish and Wildlife Service refuge biologists, regional land managers, Canadian Fish and Wildlife biologists, etc.) who have expressed interest in identifying where

proactive management can be implemented and what the barriers to implementing proactive management are across regions and management entities.

*Priority B.4.4.2:* Work with managers with complementary or spatially proximate at-risk populations to develop decision frameworks for linked decisions (i.e., actions chosen by one decision maker may affect the actions available to another decision maker). Examples would be identifying proactive management with and without importation restrictions or identifying optimal control strategies for neighboring protected area populations.

**Goal B.4.5:** Identify whether management should consider proactive, reactive, or a combination of management strategies, dependent on the presumed presence and spatial distribution of *Bsal*.

Rank: High

Rationale: A number of sampling designs may be useful for detecting the presence of the pathogen within and among populations, and work is underway to improve predictions of areas that may be at highest risk for declines should the disease be discovered. Data from a surveillance program without an associated state-dependent management plan (i.e., conditional on the state of the disease) of the appropriate scale, meaning one that matches the scale of a management decision, are of limited use; the design of an optimal program must consider the possible management responses for various scenarios. This work will be conducted in collaboration with the Surveillance & Monitoring and Research Working Groups.

Management Relevance: *Bsal* surveillance increases the likelihood of detection early in *Bsal* invasion of North America. Improving surveillance designs through the application of decision science tools can help maximize early-detection likelihoods, which increases the likelihood of a positive management response and is much more cost-effective.

Financial Needs: 360,000 USD; unfunded at present.

*Priority B.4.5.1:* Incorporate information from surveillance work into current risk assessments for *Bsal* and adjust surveillance efforts accordingly to incorporate prior expectation of *Bsal* occurrence and observations from a designed surveillance program.

*Priority B.4.5.2:* Given that previous risk assessments used a limited number of criteria to identify high risk areas, determine if other criteria can be included to improve risk assessments.



Southern Dusky Salamander (*Desmognathus auriculatus*). © Alberto López.

## 5. Surveillance & Monitoring

**Objective:** To facilitate and coordinate the surveillance of *Bsal* in North America.

**Working Group Leads:** Mike Adams (US Geological Survey, OR, USA); Jenifer Walke (Eastern Washington University, WA, USA); Olya Milenkaya (Warren Wilson College, NC, USA)

**Past Working Group Lead:** Hardin Waddle (US Geological Survey, FL, USA)

**Background:** A coordinated surveillance effort is aimed at detecting the initial introduction of *Bsal* into North America, thereby allowing for a more effective rapid response to forestall further transmission and helping to safeguard the native fauna of the continent (Framework Action 4; see Box A5.1). Management and conservation actions cannot proceed effectively without the fundamental information about when and where *Bsal* is introduced to North America. While opportunistic *Bsal* sampling improves the odds of detecting *Bsal* compared to not sampling at all (e.g., Muths et al. 2009), this haphazard approach is unlikely to detect *Bsal* at the onset of its invasion. Instead, the Surveillance & Monitoring Working Group aim is the early detection of *Bsal* to allow for an effective and rapid response, with the ultimate vision of conserving amphibian biodiversity.

Achieving a broad and robust surveillance network is difficult and expensive because of the labor involved. No single organizational entity has been identified that has this capacity. Instead, the emphasis has been on coordinating and encouraging sampling for *Bsal* by diverse partners such that something close to a reasonable level of surveillance is achieved. Initial efforts toward this goal are described below. However, because of the limitations of initial approaches, current efforts of the Surveillance & Monitoring Working Group are aimed at building an integrated network of partners in surveillance. This network will increase *Bsal* awareness, engage volunteer personnel and citizen scientists, utilize dispersed in-kind resources, and increase the amount of *Bsal* sampling.

### Past Surveillance Efforts:

**United States:** In the United States, some sampling has been conducted by federal agencies. As part of the Surveillance & Monitoring Working Group's primary objective, a one-time major

sampling effort was conducted by the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI) in 2014 to 2017 (Waddle et al. 2019, 2020). This effort included sample sites across the United States, with resources allocated according to the estimated risk of *Bsal* occurrence as per the risk assessment model results developed by Richgels et al. (2016). Over 10,000 amphibians (mostly salamanders) were sampled. *Bsal* was not detected (Waddle et al. 2019, 2020; data archived at [amphibiandisease.org](http://amphibiandisease.org) by the Data Management Working Group, see below). ARMI continues to sample at a very low level in select areas where resources allow. In addition, the US Fish and Wildlife Service has done some sampling using their National Wild Fish Health Survey funding. There is an ongoing effort to sample in Appalachia, which is one of the high-risk areas (Richgels et al. 2016).

In the United States and elsewhere, independent science investigations into *Bsal* detections from skin swabs of selected species in specific geographies are ongoing. For example, four reports of joint *Bsal* and *Bd* sampling in North America were published in Herpetological Review in 2017 (Olson 2019).

In addition to sampling, in the United States, an iNaturalist website was set up as way to gather observations from the public of sick or dead amphibians that might need follow-up investigation. Similarly, the Partners for Amphibian and Reptile Conservation (PARC) national Disease Task Team set up a Herpetofaunal Disease Alert System (HDAS; email reports to [herp\\_disease\\_alert@parcplace.org](mailto:herp_disease_alert@parcplace.org); include photograph, species affected, location, other relevant episode information, observer's name; Gray et al. 2018) that provides another way to gather observations of sick or dead amphibians that might not otherwise be reported. At its start, communication occurred between iNaturalist and HDAS, although that connection has been dormant in recent years. Reports received via HDAS or, when available, through iNaturalist, are typically forwarded to the relevant state biologist, but in some cases, when deemed necessary, members of the Disease Task Team or state authorities can use their personal networks to help facilitate further investigation.

**Canada:** In Canada, the provincial and territorial governments are the lead jurisdiction for amphibian disease surveillance. The provinces of British Columbia (BC) and Ontario have conducted the most intensive *Bd* and *Bsal* monitoring programs to date. In Ontario, over 900 amphibians were sampled opportunistically along a latitudinal gradient over a four-year period (2014–2017). All samples were tested for *Bsal*, and all tests were negative (Christina Davy, Ministry of Natural Resources and Forestry, unpubl. data). In 2016, provincial biologists in BC sampled for *Bsal* within a small number of wild Rough-skinned Newt (*Taricha granulosa*) and captive (pet store) salamander populations on the south coast—one of the high-vulnerability zones identified by Yap et al. (2015). *Bsal* was not detected by quantitative polymerase chain reaction (qPCR) analyses in any swabs from the 82 wild newts and 15 captive salamanders sampled (Govindarajulu et al. 2017). In many provinces, including Alberta, Saskatchewan, Québec and Newfoundland, the current approach is one of passive surveillance in which *Bsal* investigations are triggered by unusual or mass amphibian mortality events. However, Ontario is considering low-level opportunistic sampling over the short-term, as resources allow. In addition, amphibians seized from the illegal pet trade will be tested for *Bsal* in BC.

The Canadian public can submit reports of sick or dead amphibians to the Canadian Wildlife Health Cooperative (CWHC). The CWHC is able to advise on the collection of carcasses for follow-up investigation and screens samples for diseases and parasites to assess the health of wild populations (CWHC 2019). Canadian provinces and territories may have additional reporting tools for sick or dead amphibians, such as the Government of British Columbia's



“Frogwatching” site, which is monitored by the provincial amphibian specialist. Disease reports from Canadian locations that are received by the HDAS email address are forwarded to provincial authorities in Canada, similar to state reporting of HDAS reports received in the United States.

**Mexico:** In Mexico, surveys aimed at identifying *Bsal* in natural populations have been conducted by members of Gabriela Parra-Olea’s research laboratory at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). So far, 119 individuals of 41 species (frogs and salamanders) have been sampled, and *Bsal* has not been detected by qPCR analyses in any of the swabs (Parra-Olea, unpubl. data). Additional surveys by Eria Rebollar (Centro de Ciencias Genómicas, UNAM) and Gabriela Parra-Olea in plethodontid salamanders and *Ambystoma* species across the Trans-Mexican Volcanic Belt were planned for 2020 but had to be postponed because of COVID-19 restrictions on travel and teaching. Parra-Olea and Rebollar have begun efforts to provide certification to both of these research laboratories so that legal amphibian imports in Mexico can be screened for *Bsal*.

The Surveillance & Monitoring Working Group has strong ties to the Data Management Working Group, which helps compile *Bsal* surveillance reports and scientific studies from outside the efforts of the North American *Bsal* Task Force (Goal B.5.2). They also coordinate with the Diagnostics Working Group, which conducts *Bsal* diagnostics research, and the Response Working Group to help formulate monitoring responses if *Bsal* were to be detected. As needed, they work with the Clean Trade Working Group for Goal B.5.3 and the Decision Science Working Group, as their risk assessments have guided initial priority sampling efforts.

**Goal B.5.1:** Facilitate and support a wide-reaching, ongoing, coordinated, and sustainable *Bsal* surveillance program in Canada, Mexico, and the United States.

Rank: High

Rationale: A robust surveillance network is needed for early detection of *Bsal* upon introduction. The earlier *Bsal* is detected, the better the chance of containment and of limiting negative consequences for amphibian biodiversity.

Management Relevance: Early detection of *Bsal* in North America improves management efficacy to forestall *Bsal* spread, increasing population and species persistence likelihoods at lower financial burdens. It is a win-win strategy for retaining North American amphibian biodiversity and reducing stressors to the larger ecosystems within which amphibians are embedded.

Financial Needs: In-kind support from member agencies and institutions (especially US Geological Survey’s Amphibian Research and Monitoring Program) for personnel time, some laboratory analyses, and travel. Grant proposals are planned for partner sampling efforts, travel, and laboratory analytical costs. Estimated costs: 15,000 USD per year for the Student Network for Amphibian Pathogen Surveillance (SNAPS) portion of Priority B.5.1.1; 100,000 USD per year in 2021–2023 for initial efforts with broader surveillance in Canada, Mexico, and the United States; 200,000 USD per year for scaled-up efforts in 2023–2025.

*Priority B.5.1.1:* Establish a *Bsal* surveillance program in cooperation with colleges and universities: the Student Network for Amphibian Pathogen Surveillance (SNAPS). This priority includes development of partnership roles, a student training curriculum, sampling protocols, safety and biosecurity procedures, data procedures, and procedures to obtain necessary permits and permissions for working with wildlife and working with students within course constraints or as part of extracurricular activities.

*Priority B.5.1.2:* Facilitate and support ongoing *Bsal* surveillance in Mexico and Canada.

**Goal B.5.2:** Identify *Bsal* sampling efforts that are occurring outside of efforts coordinated by the *Bsal* Surveillance & Monitoring Working Group.

Rank: High

Rationale: Researchers and managers are conducting their own *Bsal* surveillance across North America outside of efforts spearheaded by the *Bsal* Task Force, but these efforts are not coordinated. Therefore, to maintain an ongoing account of the entire *Bsal* surveillance effort, this working group should at least contact these principal investigators (PIs) and catalogue their efforts.

Management Relevance: Multipronged, coordinated surveillance has a higher likelihood of detecting *Bsal* early upon its emergence in North America, reducing long-term management costs of *Bsal* management and protection of rare species. Coordination of efforts can reduce survey redundancies, provide better inference to North America and host taxa, and be more cost-efficient. Strategic coordination may influence independent sampling in much needed areas where gaps in effort have been revealed.

Financial Needs: In-kind support from member agencies and institutions for personnel time, some laboratory analyses, and travel. Grant proposals are planned for partner sampling efforts, travel, and laboratory analytical costs. No cost estimates are available at this time, as the workplan is still in development.

*Priority B.5.2.1:* Coordinate with the *Bsal* Research Working Group and other amphibian disease researchers across North America who are conducting *Bsal* surveillance as part of their broader research programs.

*Priority B.5.2.2:* Establish additional pathways of communication with a broader sector of amphibian biologists, permitting agencies and institutions, and diagnostic laboratories to develop a means of identifying personnel conducting *Bsal* surveillance without compromising confidential research plans.

*Priority B.5.2.3:* Encourage *Bsal* and *Bd* surveyors to input their planned, ongoing, or past efforts into the chytrid data management portal, [amphibiandisease.org](http://amphibiandisease.org).

**Goal B.5.3:** Support and facilitate sampling of amphibians in the pet trade.

Rank: High

Rationale: *Bsal* is likely to be introduced to North America through amphibian trade. Therefore, surveillance among captive amphibians is a logical priority for the early detection of *Bsal*. Furthermore, detection of *Bsal* in captive animals prior to its introduction in the wild will provide conservationists and managers with the opportunity to contain the pathogen and prevent it from affecting wild populations.

Management Relevance: Forestalling *Bsal* introduction to the wild in North America is of premier importance for retaining the integrity of North American wildlife and ecosystems, a primary goal of natural resource managers. Early *Bsal* detection in North American trade markets and captive populations (e.g., amphibians used as pets, food, and traditional medicines and populations retained in zoos, aquariums, museums, and biomedical research) can improve *Bsal* management efficacy and reduce costs of *Bsal* mitigation actions in North America.

Financial Needs: In-kind support from member agencies and institutions for personnel time, some laboratory analyses, and travel. Grant proposals are planned for partner sampling efforts, travel, and laboratory analytical costs. No cost estimates are available at this time, as the workplan is still in development.

*Priority B.5.3.1:* Coordinate with the newly established Clean Trade Working Group to support and facilitate *Bsal* sampling of amphibians in the pet trade.

*Priority B.5.3.2:* Develop a study plan for animal sampling or subsampling and eDNA water sampling for *Bsal* at ports of entry.

*Priority B.5.3.3:* Develop study plan for animal subsampling and eDNA water sampling of batches in pet stores in cooperation with state and provincial/territorial agencies.

**Goal B.5.4:** Develop initial plans for post-detection monitoring if *Bsal* were to be detected at a field or captive site in North America.

Rank: Urgent

Financial Needs: In-kind support from member agencies and institutions for personnel time.

*Priority B.5.4.1:* In cooperation with the Decision Science Working Group, partners, and existing scenario-development exercises of *Bsal* detection in different site geographic and taxonomic contexts, develop example sampling designs to detect the extent of *Bsal* at a novel detection site before mitigation actions are fully implemented.

*Priority B.5.4.2:* In cooperation with the Decision Science Working Group, partners, and existing scenario-development exercises of *Bsal* detection in different site geographic and taxonomic contexts, develop example sampling designs to detect the extent of *Bsal* at a detection site after different types of mitigation actions are fully implemented.

*Priority B.5.4.3:* To fine-tune monitoring designs at known or suspected *Bsal* detection sites, develop study plans to compare efficacy of alternative approaches; develop a priori Institutional Animal Care and Use Committee (IACUC) and state or federal permission forms and determine if expedited approvals could be gained.



Apalachicola Dusky Salamander (*Desmognathus apalachicola*). © Alberto López.

## 6. Data Management Working Group

**Objective:** To develop an online *Bsal* and *Bd* data management portal to accelerate sharing of planned or completed surveillance projects and scientific studies to accelerate the pace of learning about these pathogens tied to emerging infectious diseases and foster integrated efforts in research and monitoring across North America and among global communities.

**Working Group Leads:** Michelle Koo (AmphibiaWeb, University of California, CA, USA); Deanna H. Olson (USDA Forest Service, OR, USA)

**Background:** The Data Management Working Group is a multi-partner collaboration to retain a *Bsal* and *Bd* database of world amphibian-chytrid occurrences and projects for co-production of knowledge to improve the science and management of these pathogens. In particular, this working group works closely with other groups in the *Bsal* Task Force, the University of California at Berkeley (UC Berkeley) and its online collaborative resource [AmphibiaWeb.org](http://AmphibiaWeb.org), and the US Forest Service, manager of the world *Bd* database through 2019, in previous collaboration with Imperial College, London (Olson et al. 2013, Olson and Ronnenberg 2014). Underscoring all sources of *Bsal* and *Bd* data are the independent investigators who have been vital to compiling and disseminating *Bsal* and *Bd* surveillance results. These data are now available via development of an accessible web portal database with data import and export, analysis, and multi-database linking functions (Framework Action 5 and 8; see Box A5.1). A web portal reporting system for *Bsal* and *Bd* data provides larger-scale data management capabilities and can address novel multi-scale questions and metadata analyses across individual studies. This data-sharing capability can contribute to analyses that advance understanding of the risk of *Bsal* or *Bd* introduction to North America and assess disease risk of these potentially deadly pathogens to native amphibians in North America and elsewhere through aggregating and managing past and current disease sampling data in a common repository.

The Data Management Working Group has met its original goal by developing the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)) to comprehensively manage data on *Bd* and *Bsal*. This standards-compliant, online portal is hosted as the Disease Portal webpage on

[AmphibiaWeb.org](http://AmphibiaWeb.org), managed by UC Berkeley. The portal, which includes public and private datasets, is aimed at accelerating information sharing among global scientists, natural resource managers, and the public regarding planned and ongoing surveillance projects and scientific studies as well as the results of completed studies. Data within the portal are especially important for aiding in rapid responses and decision science for allocation of limited resources available for research and management of these amphibian emerging infectious diseases and their affected hosts. In particular, comprehensive management of *Bd* and *Bsal* detection and no-detection data by location and project is useful for development of new scientific research studies, surveillance, and effective monitoring programs and for understanding disease dynamics of chytridiomycosis. Through its website, in particular the Data Dashboard, the Amphibian Disease Portal could be an effective outreach and technical interface for the research community. Further, links to other online scientific portals, such as AmphibiaWeb, help extend the portal's reach to other audiences in education and conservation.

The Data Management Working Group is allied with the Surveillance & Monitoring, Research, Decision Science, and Outreach & Communication Working Groups, with anticipated potential interaction with the Clean Trade Working Group.

**Goal B.6.1.** Maintain comprehensive data management of *Bd* and *Bsal* samples for archived, aggregated monitoring and analytic modeling in the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)).

Rank: Urgent

Rationale: The Data Management Working Group aims to expand the scope and capacity of the new web-based Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)). In addition to increasing registered portal users and *Bsal* and *Bd* data imports, the Amphibian Disease Portal is partnering with the global bio-sampling database Geome ([geome-db.org](http://geome-db.org)) to enhance research and forecasting abilities by 1) delivering improved validation services, 2) enhancing security, and 3) improving data accessibility through third-party applications and programming tools (e.g., R statistical software and Python language applications). These developing functional capacity priorities of the web portal are described in the *Bsal* Implementation Plan (Appendix 5). Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities ([salamanderfungus.org](http://salamanderfungus.org)).

Management Relevance: The portal serves as a data management system for chytrid survey information and a communication mechanism among surveillance teams to show where efforts are planned, ongoing, or completed. This approach maximizes early detection of *Bsal* in North America and is cost effective because redundant efforts are not launched. Early detection reduces future potential costs to natural resource managers for sustaining or restoring their natural systems.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel. Grant proposals are planned for temporary hiring of web programmers to advance web user interfaces and database management procedures. Estimated costs: 30,000 USD per year for web developer to create SNAPS website for use by colleges and universities across the United States; 10,000 USD per year for *Bd* and *Bsal* data updates from the literature.

*Priority B.6.1.1:* Import *Bsal* surveillance projects and associated data into the portal as projects are planned (e.g., SNAPS, discussed by Surveillance & Monitoring Working Group above) and completed and data become available from *Bsal* Task Force partners and independent researchers.

*Priority B.6.1.2:* Develop systems and networks to identify data from *Bsal* surveillance and science reports.

*Priority B.6.1.3:* Update and upload the independent world *Bd* database from [Bd-maps.net](http://Bd-maps.net) (2007–2014) and updates to it (through 2019) developed by the Global *Bd* Mapping Project managed by the US Forest Service Pacific Northwest Research Station (Olson et al. 2021); this larger *Bd* database (2007–2019) serves as a model for *Bsal* data management, and lessons learned from *Bd* data management can improve *Bsal* data management efficiencies.

*Priority B.6.1.4:* Improve collaborations with other *Bsal* Task Force working groups, including Surveillance & Monitoring, Decision Science, Research, and Outreach & Communication, for planning of sampling efforts and coordinating outreach for improved surveillance efficiencies across North America.

**Goal B.6.2:** Advance the functional capacity of the Amphibian Disease Portal ([amphibiandisease.org](http://amphibiandisease.org)).

**Rank:** High

**Rationale:** Web portal management and improvements are dynamic as security systems adapt and technology advances, enabling broader analysis applications and weaving of multiple datasets across websites.

**Management Rationale:** Improving portal functional capacities attracts more users to the site, improving data inclusivity and expanding analytical capabilities. The result is enhanced understanding of *Bsal* current and future distributions and host taxonomic patterns of infection and disease. Such knowledge can guide management actions for *Bsal* mitigation or host species biosecurity.

**Financial Needs:** In-kind support from member agencies and institutions for personnel time. Grant proposals are planned for temporary hiring of web programmers to advance web user interfaces and database management procedures. Estimated costs: 10,000–25,000 USD per year for web-programmer upgrades; 70,000 USD for data scientist programmer for one year.

*Priority B.6.2.1:* Facilitate data import and export procedures and add new analytical applications for access.

*Priority B.6.2.2:* Expand web programming to enhance analytical capacities, e.g., incorporate interactive web displays of data and correlation charts or modeling.

*Priority B.6.2.3:* Expand integration capacity among databases, for example, to better integrate with the AmphibiaWeb portal with reciprocal links and access to geographic, habitat, or climate databases and other enhancements, such as the ability to update data with current species taxonomic information.



Marbled Salamander (*Ambystoma opacum*). © Eric Isselée.

## 7. Outreach & Communication Working Group

**Objective:** To facilitate *Bsal* communication and outreach in North America.

**Working Group Leads:** Mark Mandica (Amphibian Foundation, USA); Alex Shepack (University of Notre Dame, IN, USA)

**Past Working Group Lead:** Jillian Farkas (University of South Dakota, SD, USA)

**Background:** The Outreach & Communication Working Group produces a variety of *Bsal*-related outreach communication materials, including a web presence ([salamanderfungus.org](http://salamanderfungus.org)), fact sheets, press releases, lay and scientific articles, briefing papers, blog posts, and social media posts (Facebook and Twitter). In particular, the Outreach & Communication Working Group works with the Technical Advisory Committee (TAC) and its partners and other working groups within the *Bsal* Task Force to disseminate new *Bsal* information and products developed by the group and others. To increase the efficacy of dissemination, the Outreach & Communication Working Group continues to build an online network via social media, increasing followers and directing them to the [salamanderfungus.org](http://salamanderfungus.org) website, which serves as a hub and repository for published developments relating to issues, detections, and research regarding *Bsal*.

Although the Outreach & Communication Working Group is not tasked with conducting or publishing *Bsal* research, members help synthesize findings and communications from other groups within the *Bsal* Task Force for the purposes of producing lay articles meant to educate the public and highlighting key messages in social media posts. The scope of outreach to date has focused on national coverage in the United States, as there are salamanders at risk throughout the United States. Outreach may also be relevant for Canada and Mexico, but focused efforts outside the United States have not yet been initiated by the working group.

Finally, the Outreach & Communication Working Group organizes, designs, and publishes the annual report for the *Bsal* Task Force. This annual report summarizes advancement within all working groups and the current status of the *Bsal* fungus. This report is published on [salamanderfungus.org](http://salamanderfungus.org) and is available to both the general public and the scientific community. Goals of the working group are listed below, and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5; [salamanderfungus.org](http://salamanderfungus.org)).

**Goal B.7.1.** Work with partners to disseminate syntheses, research, and other products or activities developed by the *Bsal* Task Force via social media and newsletter articles.

Rank: High

Rationale: Build a network of partners to publish updates on *Bsal* developments and serve as an efficient mechanism for alerting the public and scientific community in the event of a positive United States detection of *Bsal*.

Management Relevance: This communication group is a dominant resource for management agency information about *Bsal*. Forging these networks of communication before *Bsal* is detected in North America will facilitate communication during a potential crisis, such as a die-off, allowing for a more measured and effective response.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel; some grant proposals are anticipated. There are no cost estimates available at this time.

*Priority B.7.1.1:* Echo *Bsal* information and key findings of new scientific research publications in new publication outlets.

*Priority B.7.1.2:* Develop publication partner relations.

*Priority B.7.1.3:* Provide outreach for new programs and operations of the *Bsal* Task Force, such as development of SNAPS with colleges and universities (see Surveillance & Monitoring, above).

*Priority B.7.1.4:* Provide media releases of *Bsal*-related information for relevant United States national or North American events.

**Goal B.7.2:** Continue to build a network on social media to communicate developments within the *Bsal* Task Force.

Rank: Urgent

Rationale: Social media is a powerful communication tool that can reach different sectors of the community. Building a network of social media partners can expand the *Bsal* Task Force purpose and aims.

Management Relevance: Outreach via social media can connect broader citizen scientists and the public to *Bsal* efforts, enhancing awareness and improving partnerships with natural resource specialists in institutions and agencies.

Financial Needs: In-kind support from member agencies and institutions for personnel time and travel; cost estimate: 2,500 USD.

*Priority B.7.2.1:* Explore utility of additional media applications for outreach and communication.

*Priority B.7.2.2:* Build social media presence.



*Priority B.7.2.3:* Summarize new information for newsletters, blogs, and social media posts.

**Goal B.7.3:** Independently, or with partners, produce public service announcements on the presence and implications of *Bsal*.

Rank: Medium

Rationale: Multimedia communication can reach broader audiences.

Management Relevance: Multimedia communication can reach agency managers and, as they are partners with common goals, be used by them for their own messaging.

Financial Needs: In-kind support from member agencies and institutions for personnel time; cost estimate: 6,000 USD.

*Priority B.7.3.1:* Work toward development of film or video outreach materials; identify videographers, develop material with the TAC, and produce videos.

*Priority B.7.3.2:* Work toward development of communication products (e.g., additional flyers, briefing papers) on *Bsal*; identify a printer, develop materials and content with the TAC, print and ship to partners.

**Goal B.7.4:** Update the *Bsal* Task Force website.

Rank: High

Rationale: The website is a go-to resource for North American *Bsal* information, including updated recent news.

Management Relevance: Natural resource managers access the website for rapid information used in their decision making.

Financial Needs: In-kind support from member agencies and institutions for personnel time; cost estimate: 3,000 USD yearly.

*Priority B.7.4.1:* Work with TAC members or partners, including the Amphibian Survival Alliance, to restructure and repopulate a more navigable website structure.

*Priority B.7.4.2:* Work with TAC members or partners, including the Amphibian Survival Alliance, to develop working group web pages and content.

*Priority B.7.4.3:* Work with TAC members or partners, including the Amphibian Survival Alliance, to develop a better *Bsal* publications list for the website and maintain updates to this list.



Spotted Dusky Salamander (*Desmognathus conanti*). © Alberto López.

## 8. Clean Trade Working Group

**Objective:** To expand efforts to forestall potential human-mediated transmission of *Bsal* into North America via trade markets.

**Working Group Lead:** Josh Jones (Pet Industry Joint Advisory Council, VA, USA)

**Background:** Although *Bsal* was first described from infections of Fire Salamanders in Europe, an Asian origin of the pathogen was implicated from the outset (Martel et al. 2013). Additional support for the hypothesis that *Bsal* evolved in Asia has been forthcoming. No lethal infections have been found there, suggesting that a long co-evolutionary history has led to resistance or tolerance by amphibian species in Asia (Laking et al. 2017). In Europe, infections were first observed in the Netherlands (Spitzen-van der Sluijs et al. 2016), followed by the discovery of *Bsal* in Belgium and Germany (Stegen et al. 2017, Lötters et al. 2020) and most recently in Spain (Martel et al. 2020). The lethal effect of *Bsal* on some European amphibian species suggests a recently arrived pathogen that has encountered naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations; possibly movement of spores by waterfowl, other wildlife, and humans; and spillover of infected individuals once held in captivity (Nguyen et al. 2017, Yuan et al. 2018). Spread of *Bsal* between continents, including the emergence of *Bsal* in Europe, is likely due to importation of infected species from locations where *Bsal* is endemic (Nguyen et al. 2017). Anurans from Asia infected with *Bsal* have been found in a pet store in Germany (Nguyen et al. 2017, Yuan et al. 2018). In addition, *Bsal* has been found on salamander species in China that are frequently imported. These findings suggest a role of trade markets in the between-continent spread of *Bsal*. In particular, the discovery that anurans can be infected opens up the possibility that trade in frogs for food, research, and pets can lead to between-continent dispersal. Clean trade measures, such as development of *Bsal* testing and certification procedures that help ensure traded animals and fomites in imports to North America are *Bsal*-free, are likely the most effective proactive measures for forestalling *Bsal* emergence in North America (Grant et al. 2017). Such measures align with Framework Action 1 (Box A5.1).

The Clean Trade Working Group was established in early 2020 in partnership with the Pet Industry Joint Advisory Council (PIJAC), and its implementation goals and priorities are under development at this time. The objective of the stakeholder-led Clean Trade Working Group is for experts in the pet trade to organize and collaborate with subject matter experts, regulators, and academics to determine what the potential components of a workable, comprehensive clean trade program for North America may include and to report these initial findings back to the *Bsal* Task

Force Technical Advisory Committee (TAC). Currently, this working group has established a network of experts in amphibian and reptile care, husbandry, and pathogen and disease research, including industry experts with knowledge of the volume and dynamics of the amphibian trade. This group has been collaborating on a regular basis to identify potential components for a comprehensive North American clean trade program to help prevent the invasion of *Bsal* to North America while allowing for the legal and responsible importation of animals for the pet trade. For example, components under discussion at this time include a description of the current status of amphibian imports; a characterization of the United States and Canada amphibian trade network; and identification of factors or processes that could reduce potential pathogen transmission, amplification, and spillover.

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